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(54) Title: SERUM BIOMARKERS IN LUNG CANCER

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-1	2011	A	IM-37	3893	A	IM-72	54026	A	IM-109	2882	B
IM-2	2030	A	IM-38	3960	A	IM-73	60170	A	IM-110	2967	B
IM-3	2089	A	IM-39	3972	A	IM-75	74372	A	IM-111	2977	B
IM-4	2128	A	IM-40	3984	A	IM-76	75545	A	IM-112	2994	B
IM-5	2146	A	IM-41	4066	A	IM-77	77543	A	IM-113	3031	B
IM-6	2186	A	IM-42	4178	A	IM-78	79507	A	IM-114	3048	B
IM-7	2232	A	IM-43	4287	A	IM-79	89854	A	IM-115	3148	B
IM-8	2277	A	IM-44	4297	A	IM-80	101831	A	IM-116	3166	B
IM-9	2295	A	IM-45	4309	A	IM-81	104301	A	IM-117	3283	B
IM-10	2318	A	IM-46	4484	A	IM-82	125160	A	IM-118	3308	B
IM-11	2411	A	IM-47	4649	A	IM-83	132976	A	IM-119	3332	B
IM-12	2434	A	IM-48	4798	A	IM-84	149099	A	IM-120	3432	B
IM-13	2467	A	IM-49	5104	A	IM-85	2018	B	IM-121	3450	B
IM-14	2482	A	IM-50	5918	A	IM-86	2029	B	IM-122	3561	B
IM-15	2498	A	IM-51	6122	A	IM-87	2144	B	IM-123	3615	B
IM-16	2565	A	IM-52	6192	A	IM-88	2130	B	IM-124	3714	B
IM-17	2574	A	IM-53	6452	A	IM-89	2168	B	IM-125	3730	B
IM-18	2586	A	IM-54	6660	A	IM-90	2184	B	IM-126	3834	B
IM-19	2605	A	IM-55	7768	A	IM-91	2200	B	IM-127	3899	B
IM-20	2722	A	IM-56	8145	A	IM-92	2284	B	IM-128	3969	B
IM-21	2746	A	IM-57	8954	A	IM-93	2299	B	IM-129	3986	B
IM-22	2788	A	IM-58	9312	A	IM-94	2314	B	IM-130	3997	B
IM-23	2855	A	IM-59	9449	A	IM-95	2414	B	IM-131	4013	B
IM-24	2871	A	IM-60	10272	A	IM-96	2428	B	IM-132	4181	B
IM-25	2984	A	IM-61	11663	A	IM-97	2451	B	IM-133	4297	B
IM-26	3030	A	IM-62	13376	A	IM-98	2468	B	IM-134	4311	B
IM-27	3144	A	IM-63	14698	A	IM-99	2483	B	IM-135	4465	B
IM-28	3243	A	IM-64	15190	A	IM-100	2565	B	IM-136	4484	B
IM-29	3273	A	IM-64	66758	A	IM-101	2583	B	IM-137	4579	B
IM-30	3290	A	IM-65	15951	A	IM-102	2597	B	IM-138	4608	B
IM-31	3369	A	IM-66	15172	A	IM-103	2697	B	IM-139	4669	B
IM-32	3445	A	IM-67	15925	A	IM-104	2715	B	IM-140	4747	B
IM-33	3483	A	IM-68	23436	A	IM-105	2740	B	IM-141	4862	B
IM-34	3676	A	IM-69	39794	A	IM-106	2752	B	IM-142	4891	B
IM-35	3779	A	IM-70	44166	A	IM-107	2767	B	IM-143	5033	B
IM-36	3793	A	IM-71	46890	A	IM-108	2865	B	IM-144	5077	B

(57) Abstract: Certain biomarkers and biomarker combinations are useful in a qualifying lung cancer status in a subject. A diagnostic methodology employing these biomarkers and combinations can detect whether a subject has lung cancer.

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SERUM BIOMARKERS IN LUNG CANCER

BACKGROUND OF THE INVENTION

[0001] The present invention relates generally to the field of serum biomarkers in lung carcinoma. More particularly, the invention relates to serum biomarkers that can distinguish lung cancer from normal.

[0002] Lung cancer is the leading cause of cancer death worldwide, resulting in 150,000 deaths per year in the United States. The mortality rate from lung cancer is greater than the combined mortality from breast, prostate and colorectal cancers. On the basis of morphology, lung cancer can be broadly classified into four main categories namely, adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma. In Hong Kong from 1990 to 1996, the proportions for adenocarcinoma, squamous cell carcinoma, large cell undifferentiated carcinoma and small cell carcinoma are 45.5%, 27.5%, 4.7% and 10.3% respectively. Both squamous cell carcinoma and small cell carcinoma are strongly associated with a smoking history.

[0003] Adenocarcinoma, squamous cell carcinoma, and large cell undifferentiated carcinoma are usually referred as "non-small cell carcinoma." They are relatively chemo-resistant, and hence the mainstay of treatment is surgery. By contrast, small cell carcinoma has a higher propensity for distant metastases and is mainly treated by chemotherapy.

[0004] Biopsy can be used to diagnose lung cancer, but it is an invasive procedure and, therefore, less than desirable. Other diagnostic methods for lung cancer include ultrasound and computed tomography (CT) scan.

[0005] It would be highly desirable to have a biomarker or combination of biomarkers capable of distinguishing between lung cancer and normal cells. In addition, a simple test could aid in tracking treatment progress and even identify molecular targets for therapy. The literature on lung cancer diagnosis has not disclosed heretofore such a biomarker or combination of biomarkers, however.

SUMMARY OF THE INVENTION

[0006] In accordance with the present invention, biomarkers and combinations of biomarkers are used to identify lung cancer. The method successfully distinguishes between lung cancer and normal states, and can be used to identify the particular type of lung cancer. In one embodiment, a method for qualifying lung carcinoma status in a subject (e.g., a patient) comprises analyzing a biological sample from the subject for one or more of the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B. Thus, to assess overall lung cancer risk versus normal, a biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310,

[0007] wherein the biomarker is differentially present in samples of a subject with lung cancer and a so-called "normal" subject that is free of lung cancer.

[0008] More preferably, one or more of the top 15 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. Thus, for assessing overall lung cancer status versus normal, the protein is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-471, IM-510, IM-544, IM-474, and IM-155, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70.

[0009] Still more preferably, one or more of the top 5 biomarkers as shown in Figure 2 or Figures 4A and 4B is used to qualify lung cancer status. In this instance, for overall lung cancer status versus normal, the biomarker is selected from the group consisting of

(A) IM-522, IM-273, IM-520, IM-519, and IM-454, or

(B) WM-61, WM-447, WM-446, WM-133, and WM-119.

[0010] In one embodiment, the method measures a plurality of biomarkers. The plurality of biomarkers can be measured simultaneously.

[0011] Biomarkers that, by themselves, are able to identify lung cancer include the WM-446 and WM-447 protein biomarkers, and these are particularly preferred.

[0012] The present invention also provides a method for qualifying lung cancer status in a subject (e.g., a patient), comprising (A) providing a spectrum generated by subjecting a biological sample from said subject to mass spectroscopic analysis that includes profiling on a chemically-derivatized affinity surface, and (B) putting the spectrum through pattern-recognition analysis that is keyed to at least one peak selected from the top 50 biomarkers as shown in Figure 2 or Figures 4A and 4B. Thus, for qualifying overall lung cancer status, the biomarker is selected from the, group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268, or

(B) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

[0013] For assessing the overall lung cancer status, the pattern-recognition analysis may, for example, be paired to a pair of peaks selected from the group consisting of

(A) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM- 266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or

(B) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

[0014] More preferably, for assessing overall lung cancer status, the pattern-recognition analysis is keyed to a pair of peaks selected from the group consisting of

(A) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or

(B) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

[0015] Alternatively, the pattern-recognition analysis for assessing overall lung cancer status may be keyed to a triplet of peaks selected from the group consisting of

(A) IM-266, IM-454 and IM-474; and IM-266, IM-474 and IM-544;

or

(B) WM-447, WM-19 and WM-473.

[0016] In other embodiments, the pattern-recognition analysis may be keyed to a combination of more than three peaks, more particularly to a combination of 4, 5 or 6 peaks, where the combination is selected from among the combinations shown in Tables 1 and 2 herein.

[0017] In each case, the biomarker is differentially present in samples of a subject with lung cancer and a normal subject.

[0018] The invention also contemplates a kit for detecting and diagnosing lung cancer, thereby to assess lung cancer status. Kits within the invention comprise, for example, (i) an adsorbent attached to a substrate that retains one or more of the biomarkers shown in Figure 2 or Figures 4A and 4B, and (ii) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent. An inventive kit may further comprise a washing solution and/or instructions for making a washing solution. The kits may include more than type of adsorbent, each present on a different substrate, *e.g.*, on a WCX and IMAC biochip. In addition, the kits may comprise one or more containers with biomarker samples, to be used as standard(s) for calibration. The substrate comprising the adsorbent may be designed to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry, preferably mass spectrometry. Alternatively, the kit may further comprise a second substrate adapted to engage the probe interface, on which the substrate comprising the adsorbent is mounted.

[0019] The method and kit according to the invention produce an article of manufacture in which one or more biomarkers according to the invention are bound to an adsorbent, optionally contacted with a matrix or energy absorbing molecule.

[0020] The present invention also provides software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers according to the invention. In one embodiment, the algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers. In another embodiment, the algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers. In yet another embodiment, the algorithm

comprises an artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

[0021] In certain embodiments, the present invention provides methods and kits that use serum amyloid a protein or a fragment thereof to qualify lung carcinoma status in a subject. In one of these embodiments, the serum amyloid a biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons. In another embodiment, the serum amyloid a biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons. In yet another embodiment, the serum amyloid a biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figures 1A-1D show all biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0023] Figure 2 shows the top 50 biomarkers identified with a Cu(II) IMAC3 ProteinChip® array format.

[0024] Figures 3A-3O show all biomarkers identified with a WCX ProteinChip® array format.

[0025] Figures 4A and 4B show the top 50 biomarkers identified with a WCX ProteinChip® array format.

[0026] Figure 5 shows fragments of serum amyloid A (SAA) that are biomarkers according to the present invention.

[0027] Figure 6 shows identification of SAA biomarkers with an anti-SAA antibody.

[0028] Figures 7-16 are spectra from WCX chips in which all of the top 15 WCX marker peaks are labeled, along with various other peaks from among the top 50 WCX peaks. Red shows spectra from lung cancer patients and gray shows normals.

[0029] Figures 17-28 are spectra from IMAC chips in which all of the top 15 WCX marker peaks are labeled, along with various other peaks from among the top 50 IMAC peaks. Blue shows spectra from lung cancer patients and gray shows normals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0030] In accordance with the present invention, a series of biomarkers associated with lung cancer has been discovered. In the present context, a biomarker is an organic biomolecule, particularly a polypeptide or protein, which is differentially present in a sample taken from a subject having lung cancer as compared to a comparable sample taken from a normal subject. A biomarker also may be differentially present in a sample taken from a subject with one type of lung cancer, *e.g.*, small cell carcinoma, as compared to a comparable sample taken from a subject with a different type of lung cancer, *e.g.*, adenocarcinoma or squamous cell carcinoma, or differentially present at different stages of a type of lung cancer. A biomarker is differentially present in samples taken from two groups of subjects if it is present at an elevated level or a decreased level in samples of the first group as compared to samples of the second group. More particularly, a biomarker is a polypeptide that is characterized by an apparent molecular weight, as determined by mass spectrometry, and that is present in samples from lung cancer subjects in an elevated or decreased level, as compared to subjects that do not have lung cancer. A biomarker is differentially present between two sets of samples if the amount of the biomarker in one sample set differs in a statistically significant way ($p < 0.01$) from the amount of biomarker in the other sample set.

[0031] The biomarkers of the invention can be used to assess lung cancer status in a subject. For example, they are capable of identifying lung cancer and successfully distinguishing it from normal subjects, thereby providing a way of diagnosing the presence or absence of lung cancer, including the presence or absence of a particular kind of lung cancer. In addition, the biomarkers are useful in assessing the risk of developing lung cancer, in staging of lung cancer and in assessing the effectiveness of treatment. Thus, "lung cancer status" in the context of the present invention includes, *inter alia*, the presence or absence of disease, the risk of developing disease, the stage of the disease, and the effectiveness of treatment of disease. Based on this status, further procedures may be indicated, including additional diagnostic tests or therapeutic procedures or regimens, such as endoscopy, biopsy, surgery, chemotherapy, immunotherapy, and radiation therapy.

[0032] In some instances, a single biomarker is capable of identifying lung cancer with a sensitivity or specificity of at least 85%, whereas, in other instances, a combination or plurality of biomarkers is used to obtain a sensitivity or specificity of at least 85%. The biomarkers and combinations of biomarkers thus can be used to qualify lung cancer status in a subject or patient.

[0033] The biomarkers according to the invention are present in serum. The biological sample used according to the present invention, however, need not be a serum sample. Thus, a biological sample for qualifying lung cancer status may be a serum, plasma or blood sample, although serum samples are preferred.

[0034] All of the biomarkers are characterized by molecular weight. A list of all the biomarkers obtained with the Cu(II) IMAC3 ProteinChip® array (Ciphergen Biosystems, Inc., Fremont, California, USA) is provided in Figures 1A-1D, and Figure 2 lists the top 50 biomarkers that distinguish between lung cancer and normal subjects that are identified by Cu(II) IMAC3 protocol described herein. Figures 3A-3O comprise a list of all the biomarkers obtained with the WCX2 ProteinChip® array, and Figures 4A and 4B comprise a ranking of the top 50 biomarkers that distinguish between (i) lung cancer and normal subjects, (ii) subjects with each of four types of lung cancer and normal subjects, and (iii) two types of lung cancer, *e.g.*, adenocarcinoma versus squamous cell carcinoma, as identified by WCX2 protocol described herein.

[0035] The top 50 biomarkers were determined by decision tree analysis using Biomarker Patterns™ software from Ciphergen Biosystems, Inc. Biomarkers other than those within the top 50 also are useful in distinguishing between subjects with lung cancer and normal subjects and may, in particular, appear in decision trees with multiple nodes. In preferred embodiments, one or more of the top 15 biomarkers are used, and in even more preferred embodiments, one or more of the top 5 biomarkers are used.

[0036] In each of Figures 1A-1D and 3A-3O, the number in the first column is the biomarker identifier. Thus, the first row in Figures 1A-1D relates to biomarker IM-1, the second row relates to biomarker IM-2, and so forth ("IM-" denoting biomarkers identified with the IMAC chip). Similarly, the first row in Figures 3A-3O relates to

biomarker WM-1 and the second row relates to biomarker WM-2 ("WM-" denoting biomarkers identified with the WCX2 chip). The number in the second column in Figures 1A-1D is the apparent molecular weight of the biomarker in daltons, as determined by mass spectrometry. In Figures 3A-3O, the apparent molecular weights for the biomarkers identified in the first column are reported in columns 3 through 11. The letter in the second column of Figures 1A-1D and the third column of Figures 3A-3O denotes the fraction in which the biomarker elutes in the protocol described herein; that is, biomarkers with an "A" elute in the first fraction, biomarkers with a "B" elute in the second fraction, and so forth. The fraction in which the biomarker elutes correlates with its pI, which biomarkers eluting at higher pH having a higher pI, and biomarkers eluting at lower pH having a lower pI.

[0037] Presenting the mass and affinity characteristics of a given biomarker within the invention, as in this description, characterizes that biomarker so as allow one to obtain and measure it, in accordance with the teachings herein. If desired, any of the biomarkers can be sequenced, in order to obtain an amino acid sequence, but this is not required to practice the present invention.

[0038] For example, a biomarker can be peptide mapped with a number of enzymes, such as trypsin and V8 protease, and the molecular weights of the digestion fragments can be used to search databases for sequences that match the molecular weights of the digestion fragments generated by the various enzymes. Alternatively, if the biomarkers are not proteins included in known databases, degenerate probes can be made based on the N-terminal amino acid sequence of the biomarker, which then are used to screen a genomic or cDNA library created from a sample from which the biomarker was initially detected. The positive clones can be identified, amplified, and their recombinant DNA sequences can be subcloned using techniques which are well known. Finally, protein biomarkers can be sequenced using protein ladder sequencing. Protein ladders can be generated by fragmenting the molecules and subjecting fragments to enzymatic digestion or other methods that sequentially remove a single amino acid from the end of the fragment. The ladder is then analyzed by mass spectrometry. The difference in masses of the ladder fragments identifies the amino acid removed from the end of the molecule.

[0039] Several biomarkers identified in accordance with the teachings of the present invention fit to serum amyloid A (SAA) or to a fragment of SAA. SAA is a well-known acute phase inflammatory marker. A number of the SAA biomarkers are identified in Figure 5 by both molecular mass and amino acid sequence. Most of these markers bound anti-SAA antibodies, as shown in Figure 6. The intact mass of SAA is 11.5 to 11.7 kD, and these biomarkers also have been identified by the present methodology.Fragments preferably have a molecular mass of at least about 200 Daltons, more preferably at least about 500 Daltons. In even more preferred embodiments, fragments have a molecular mass of at least about 800 Daltons, and most preferably at least about 1 Kilodalton.

[0040] In one embodiment, the fragments of SAA include a sequence of amino acids that is recognized by an epitope of an anti-SAA antibody.. One way of identifying suitable fragments for use in the present invention is to enzymatically digest SAA and test the resulting fragments for the ability to bind to an anti-SAA antibody. Fragments that bind anti-SAA antibody can be sequenced using techniques well-known in the art, although the sequence of the fragment is not needed to practice the invention. In order to practice the invention with a fragment from the enzymatic digest that is identified as binding anti-SAA antibody, all that is required is to subject to the fragment to mass spectrometry to determine its mass.

[0041] The serum biomarkers according to the present invention were identified by comparing mass spectra of samples derived from sera from two groups of newly-diagnosed subjects, subjects with lung cancer and normal subjects. The subjects were diagnosed according to standard clinical criteria. Lung cancer subjects were histologically confirmed, and subjects without lung cancer were followed for at least 18 months following serum collection for any sign of lung cancer, to exclude subjects with asymptomatic lung cancer.

[0042] Sera from each group of subjects was collected, and fractionated with Q Ceramic HyperDF ion exchange resin (Biosepra SA, France) into six fractions which eluted at different pH. Fraction A comprised the flow through plus pH 9 eluant, Fraction B comprised the pH 7 eluant, Fraction C comprised the pH 5 eluant, Fraction D comprised the pH 4 eluant, Fraction E comprised the pH 3 eluant, and Fraction F

comprised isopropyl alcohol/acetonitrile TFA eluant. Fractions A through F are identified on Figures 7-28 as Fractions 1 through 6, respectively.

[0043] Each fraction was diluted and applied to a ProteinChip® array, either a Cu(II) IMAC3 or WCX2 chip array. Both of these chip arrays are produced by Ciphergen Biosystems, Inc. (Fremont, CA).

[0044] The Cu(II) IMAC3 is an "immobilized metal affinity-capture" chip, with a nitrilotriacetic acid surface for high-capacity copper binding and subsequent affinity capture of proteins with metal binding residues. Imidazole may be used in binding and washing solutions to moderate protein binding, including binding of non-specific proteins. Increasing the concentration of imidazole in the washing buffers reduces the binding of the target proteins. It is produced by photopolymerizing 5-methylacylamido-2-(N,N-biscarboxymethylamino)pentanoic acid (7.5 wt%) and N,N'-methylenebisacrylamide (0.4 wt%) using (-) riboflavin (0.02 wt%) as a photoinitiator. The monomer solution is deposited onto the chip substrate and irradiated to photopolymerize. The chip then is activated with Cu(II).

[0045] The WCX2 is a weak cation exchange array with a carboxylate surface to bind cationic proteins. The negatively charged carboxylate groups on the surface of the WCX2 chip interact with the positive charges exposed on the target proteins. The binding of the target proteins is reduced by increasing the concentration of salt or by increasing the pH of the washing buffers.

[0046] Following application of the eluant fraction, the chips were incubated to allow the polypeptides in the eluant to bind to the sites on the chip by an affinity interaction. After incubation, each chip array was washed to remove polypeptides that bind non-specifically and buffer contaminants. That chip then was dried, and an energy absorbing molecule or matrix was applied to it, to facilitate desorption and ionization in a mass spectrometer.

[0047] In the mass spectrometer, retained polypeptides were desorbed from the chip array by laser desorption and ionization in a ProteinChip® Reader, which is integrated with ProteinChip® Software and a personal computer to analyze proteins captured on chip arrays. The ion optic and laser optic technologies in the ProteinChip® Reader detects proteins ranging from small peptides of less than 1000 Da up to proteins of

300 kilodaltons or more, and calculates the mass based on time-of-flight. Ionized polypeptides were detected and their mass accurately determined by this Time-of-Flight (TOF) Mass Spectrometry.

[0048] The mass spectra obtained for each group were subjected to scatter plot analysis, to eliminate run-to-run variation. Protein clusters on the scatter plot that had the same pattern for both lung cancer and normal subjects, *i.e.*, protein clusters that were either elevated in both groups of subjects or depressed in both groups of subjects, were eliminated as potential biomarkers. The remaining polypeptides were further analyzed for their ability to accurately identify subjects with lung cancer. Because the molecular weights were derived from scatter plot analysis, and because of limits on the ability of mass spectrometry to resolve molecular weights, the "absolute" molecular weight values given in Figures 1A-1D and 3A-3O actually represent approximate molecular weights.

[0049] The biomarkers of this invention are characterized by their mass-to-charge ratio as determined by mass spectrometry. The mass-to-charge ratio of each biomarker is provided in Figures 1A-1D and 3A-3O. For example, IM-1 in Figure 1A has a measured mass-to-charge ratio of 2011. The mass-to-charge ratios were determined from mass spectra generated on a Ciphergen Biosystems, Inc. PBS II mass spectrometer. This instrument has a mass accuracy of about +/- 0.15 percent. Additionally, the instrument has a mass resolution of about 400 to 1000 m/dm, where m is mass and dm is the mass spectral peak width at 0.5 peak height. The mass-to-charge ratio of the biomarkers was determined using Biomarker Wizard™ software (Ciphergen Biosystems). Biomarker Wizard assigns a mass-to-charge ratio to a biomarker by clustering the mass-to-charge ratios of the same peaks from all the spectra analyzed, as determined by the PBSII, taking the maximum and minimum mass-to-charge-ratio in the cluster, and dividing by two. Accordingly, the masses provided reflect these specifications.

[0050] The biomarkers of this invention are further characterized by the shape of their spectral peak in time-of-flight mass spectrometry. Mass spectra showing peaks representing the biomarkers are presented in Figures 7-28. The biomarker identifier numbers from Figures 2 and 4A-4B, respectively, are shown next to the peak, along

with their rank, which is indicated in parentheses below the biomarker identifier number.

[0051] The biomarkers of this invention are further characterized by their binding properties on chromatographic surfaces. Most of the biomarkers bind to IMAC (Cu) or WCX adsorbents (e.g., the Ciphergen® IMAC (Cu) or WCX ProteinChip® arrays) after washing as described herein.

[0052] Thus, a given molecular weight for a biomarker herein should be interpreted as the midpoint of a molecular-weight range. The accuracy of the mass spectrometer is +/- 0.15%, and the actual molecular weight for a biomarker is therefore the value given, +/- 0.15%. For example, the actual molecular weight for biomarker IM-273 is 11705 +/- 0.15%, or between 11687 and 11722. Often, the range surrounding the "absolute" value given in the figure is no more than +/- 5 daltons (2006 to 2016 for IM-1), generally no more than +/- 3 daltons (2008 to 2014 for IM-1), and often as small as +/- 1 dalton (2010 to 2012 daltons for IM-1).

[0053] CART® (Salford Systems, San Diego, CA), a classification and regression tree software, was used to determine whether a potential biomarker had predictive value in assessing lung cancer. A software macro randomly selected a subset of 15% of the peaks from Figures 1A-1D or Figures 3A-3O. The peaks and peak heights from each sample were provided to the CART® software for analysis. The software performed an iterative analysis until a single decision tree was generated that was capable of distinguishing between cancerous and non-cancerous. Each node in the resulting decision tree sorted based on the peak height of a single biomarker. A tree may contain any number of nodes, but generally contains from 1 to 6 nodes. From a practical standpoint in a commercial diagnostic test, a decision tree with fewer nodes is preferred. A total of 2000 decision trees, each based on a different 15% subset of the peaks from Figures 1A-1D or Figures 3A-3O, were generated.

[0054] The CART® software assigned a score to each biomarker in the subset, based on its relative importance. A score of 100 is very high and a score of 0 is very low. The CART® software also determined the sensitivity and specificity of each decision tree.

[0055] The data generated by the decision tree analysis was subjected to further analysis. The biomarkers were ranked based on their average scores, which were determined by adding up a biomarker's scores for each decision tree in which it appeared, and dividing by the total number of decision trees in which the biomarker appeared. Approximately 500 of the potential biomarkers showed up in at least one tree, and most of the biomarkers showed up in about 150 to 400 of the two thousand trees. The top 50 biomarkers for the IMAC and WCX chip arrays as determined by this method are shown in Figures 2 and 4A-4B, respectively.

[0056] All of the trees having sensitivities and specificities greater than 85% also were identified. All trees capable of distinguishing lung cancer from normal and having from 1 to 6 nodes that meet the 85/85 criterion are shown in Tables 1 and 2.

TABLE 1. Decision trees with IMAC Biomarkers.

2 Nodes				
474	151			
474	156			
522	507		2 trees	
522	440		2 trees	
3 Nodes				
266	454	474		
474	156	153		
474	40	156		
520	276	113		
520	265	401		
522	151	474		
522	478	153		
522	156	474		
4 Nodes				
148	521	508	251	

266	544	474	493	
266	157	126	420	
266	544	474	482	
266	471	474	38	
266	544	474	38	
266	514	471	203	
522	58	266	474	
5 Nodes				
266	544	473	151	437
266	454	474	153	264
273	143	544	401	199

TABLE 2. Decision Trees with WCX Biomarkers.

1 Node					
446					
447					
2 Nodes					
282	127				
3 Nodes					
61	16	27			
61	119	154			
61	120	154			
61	369	184			
61	184	129			
61	19	282			
133	282	319			
282	59	218			
282	111	65			

446	19	16			
4 Nodes					
61	369	282	184		
61	48	203	3		
446	369	111	67		
446	466	58	120		
446	19	59	113		
446	282	19	47		
447	118	59	417		
447	118	59	473		
447	65	59	275		
447	19	59	282		
447	369	59	206		
447	19	59	253		
447	19	47	70		
5 Nodes					
61	369	128	184	197	
61	17	425	366	341	
133	139	363	216	273	
282	133	48	19	253	
369	310	19	109	384	
446	282	15	319	66	
447	19	71	473	31	
447	19	17	473	438	
447	47	31	365	59	
6 Nodes					
369	366	192	471	19	439

[0057] Each of the biomarker combinations of Tables 1 and 2 are preferred combinations for distinguishing lung cancer subjects from normal subjects in accordance with the present invention.

[0058] All biomarkers that appeared in at least two of the trees that met the 85/85 criterion were identified. For these biomarkers, Tables 3 and 4 provide the number of times the biomarker occurred in a trees that met the criterion, as well as the ranking of that biomarker on the top 50 lists of Figures 2 and 4A-4B.

TABLE 3. Correlation of IMAC biomarker decision tree frequencies and ranking.

Peak		# times		Rank
266		9		9
522		8		1
474		4		14
520		2		3
148		1		8
273		1		2

TABLE 4. Correlation of WCX biomarker decision tree frequencies and ranking.

Peak		# times		Rank
447		11		2
61		10		1
446		7		3
282		4		9
369		2		8
133		2		4

[0059] Biomarkers that occurred frequently in the highly discriminatory trees occurred among the top 50 ranked biomarkers, and typically had a top 10 ranking. In addition, certain pairs of biomarkers reappear, *e.g.*, WM-447 and WM-59, WM-447 and WM-19, WM-19 and WM-59, IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-522 and IM-266. There also are repeats among triplets of biomarkers, such as IM-266, IM-266 and IM-38, and WM-447, WM-19 and WM-473. Other repeating pairs and trios of biomarkers can be seen in Tables 3 and 4, and are preferred.

[0060] Biomarkers and combinations of biomarkers identified in accordance with the present description may be used to qualify lung cancer status in a subject. In particular, a biomarker or combination of biomarkers can be used to distinguish lung cancer patients from normal patients with a high degree of specificity or sensitivity, *i.e.,* greater than at least 85%, preferably greater than at least 90%, and more preferably greater than 95%.

[0061] According to one aspect of the invention, therefore, the detection of biomarkers for diagnosis of lung cancer status entails contacting a sample from a subject with a substrate, e.g., a SELDI probe, having an adsorbent thereon, under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, for example, mass spectrometry. Other detection paradigms that can be employed to this end include optical methods, electrochemical methods (voltametry and amperometry techniques), atomic force microscopy, and radio frequency methods, *e.g.*, multipolar resonance spectroscopy. Illustrative of optical methods, in addition to microscopy, both confocal and non-confocal, are detection of fluorescence, luminescence, chemiluminescence, absorbance, reflectance, transmittance, and birefringence or refractive index (e.g., surface plasmon resonance, ellipsometry, a resonant mirror method, a grating coupler waveguide method or interferometry).

[0062] In one aspect, the markers of this invention are detect by gas phase ion spectrometry, which refers to the use of a gas phase ion spectrometer to detect gas phase ions. A gas phase ion spectrometer is an apparatus that detects gas phase ions. Gas phase ion spectrometers include an ion source that supplies gas phase ions. Gas

phase ion spectrometers include, for example, mass spectrometers, ion mobility spectrometers, and total ion current measuring devices.

[0063] "Mass spectrometer" refers to a gas phase ion spectrometer that measures a parameter which can be translated into mass-to-charge ratios of gas phase ions. Mass spectrometers generally include an ion source and a mass analyzer. Examples of mass spectrometers are time-of-flight, magnetic sector, quadrupole filter, ion trap, ion cyclotron resonance, electrostatic sector analyzer and hybrids of these. "Mass spectrometry" refers to the use of a mass spectrometer to detect gas phase ions. "Laser desorption mass spectrometer" refers to a mass spectrometer which uses laser as a means to desorb, volatilize, and ionize an analyte.

[0064] "Mass analyzer" refers to a sub-assembly of a mass spectrometer that comprises means for measuring a parameter which can be translated into mass-to-charge ratios of gas phase ions. In a time-of flight mass spectrometer the mass analyzer comprises an ion optic assembly, a flight tube and an ion detector.

[0065] "Ion source" refers to a sub-assembly of a gas phase ion spectrometer that provides gas phase ions. In one embodiment, the ion source provides ions through a desorption/ionization process. Such embodiments generally comprise a probe interface that positionally engages a probe in an interrogatable relationship to a source of ionizing energy (e.g., a laser desorption/ionization source) and in concurrent communication at atmospheric or subatmospheric pressure with a detector of a gas phase ion spectrometer.

[0066] Forms of ionizing energy for desorbing/ionizing an analyte from a solid phase include, for example: (1) laser energy; (2) fast atoms (used in fast atom bombardment); (3) high energy particles generated via beta decay of radionucleides (used in plasma desorption); and (4) primary ions generating secondary ions (used in secondary ion mass spectrometry). The preferred form of ionizing energy for solid phase analytes is a laser (used in laser desorption/ionization), in particular, nitrogen lasers, Nd-Yag lasers and other pulsed laser sources. "Fluence" refers to the laser energy delivered per unit area of interrogated image. Typically, a sample is placed on the surface of a probe, the probe is engaged with the probe interface and the probe

surface is struck with the ionizing energy. The energy desorbs analyte molecules from the surface into the gas phase and ionizes them.

[0067] Other forms of ionizing energy for analytes include, for example: (1) electrons which ionize gas phase neutrals; (2) strong electric field to induce ionization from gas phase, solid phase, or liquid phase neutrals; and (3) a source that applies a combination of ionization particles or electric fields with neutral chemicals to induce chemical ionization of solid phase, gas phase, and liquid phase neutrals.

[0068] A preferred mass spectrometric technique for use in the invention is Surface Enhanced Laser Desorption and Ionization (SELDI), as described, for example, in U.S. patents No. 5,719,060 and No. 6,225,047, both to Hutchens and Yip, in which the surface of a probe that presents the analyte (here, one or more of the biomarkers) to the energy source plays an active role in desorption/ionization of analyte molecules. In this context, "probe" refers to a device adapted to engage a probe interface and to present an analyte to ionizing energy for ionization and introduction into a gas phase ion spectrometer, such as a mass spectrometer. A probe typically includes a solid substrate, either flexible or rigid, that has a sample-presenting surface, on which an analyte is presented to the source of ionizing energy.

[0069] One version of SELDI, called Surface-Enhanced Affinity Capture" or "SEAC," involves the use of probes comprised of a chemically selective surface ("SELDI probe"). A "chemically selective surface" is one to which is bound either the adsorbent, also called a "binding moiety" or "capture reagent," or a reactive moiety that is capable of binding a capture reagent, *e.g.*, through a reaction forming a covalent or coordinate covalent bond.

[0070] The phrase "reactive moiety" here denotes a chemical moiety that is capable of binding a capture reagent. Epoxide and carbodiimidizole are useful reactive moieties to covalently bind polypeptide capture reagents such as antibodies or cellular receptors. Nitriloacetic acid and iminodiacetic acid are useful reactive moieties that function as chelating agents to bind metal ions that interact non-covalently with histidine containing peptides. A "reactive surface" is a surface to which a reactive moiety is bound. An "adsorbent" or "capture reagent" can be any material capable of

binding a biomarker of the invention. Suitable adsorbents for use in SELDI, according to the invention, are described in U.S. patent No. 6,225,047, *supra*.

[0071] One type of adsorbent is a "chromatographic adsorbent," which is a material typically used in chromatography. Chromatographic adsorbents include, for example, ion exchange materials, metal chelators, immobilized metal chelates, hydrophobic interaction adsorbents, hydrophilic interaction adsorbents, dyes, simple biomolecules (e.g., nucleotides, amino acids, simple sugars and fatty acids), mixed mode adsorbents (*e.g.*, hydrophobic attraction/electrostatic repulsion adsorbents). "Biospecific adsorbent" is another category, for adsorbents that contain a biomolecule, *e.g.*, a nucleotide, a nucleic acid molecule, an amino acid, a polypeptide, a polysaccharide, a lipid, a steroid or a conjugate of these (*e.g.*, a glycoprotein, a lipoprotein, a glycolipid). In certain instances the biospecific adsorbent can be a macromolecular structure such as a multiprotein complex, a biological membrane or a virus. Illustrative biospecific adsorbents are antibodies, receptor proteins, and nucleic acids. A biospecific adsorbent typically has higher specificity for a target analyte than a chromatographic adsorbent.

[0072] Another version of SELDI is Surface-Enhanced Neat Desorption (SEND), which involves the use of probes comprising energy absorbing molecules that are chemically bound to the probe surface ("SEND probe"). The phrase "Energy absorbing molecules" (EAM) denotes molecules that are capable of absorbing energy from a laser desorption ionization source and, thereafter, contributing to desorption and ionization of analyte molecules in contact therewith. The EAM category includes molecules used in MALDI, frequently referred to as "matrix," and is exemplified by cinnamic acid derivatives, sinapinic acid (SPA), cyano-hydroxy-cinnamic acid (CHCA) and dihydroxybenzoic acid, ferulic acid, and hydroxyaceto-phenone derivatives. The category also includes EAMs used in SELDI, as enumerated, for example, by U.S. 5,719,060 and U.S. 60/351,971 (Kitagawa), filed January 25, 2002.

[0073] Another version of SELDI, called Surface-Enhanced Photolabile Attachment and Release (SEPAR), involves the use of probes having moieties attached to the surface that can covalently bind an analyte, and then release the analyte through breaking a photolabile bond in the moiety after exposure to light, *e.g.*, to laser light.

For instance, see U.S. 5,719,060. SEPAR and other forms of SELDI are readily adapted to detecting a biomarker or biomarker profile, pursuant to the present invention.

[0074] The detection of the biomarkers according to the invention can be enhanced by using certain selectivity conditions, *e.g.*, adsorbents or washing solutions. The phrase "wash solution" refers to an agent, typically a solution, which is used to affect or modify adsorption of an analyte to an adsorbent surface and/or to remove unbound materials from the surface. The elution characteristics of a wash solution can depend, for example, on pH, ionic strength, hydrophobicity, degree of chaotropism, detergent strength, and temperature.

[0075] Pursuant to one aspect of the present invention, a sample is analyzed by means of a "biochip," a term that denotes a solid substrate, having a generally planar surface, to which a capture reagent (adsorbent) is attached. Frequently, the surface of a biochip comprises a plurality of addressable locations, each of which has the capture reagent bound there. A biochip can be adapted to engage a probe interface and, hence, function as a probe in gas phase ion spectrometry preferably mass spectrometry. Alternatively, a biochip of the invention can be mounted onto another substrate to form a probe that can be inserted into the spectrometer.

[0076] A variety of biochips is available for the capture of biomarkers, in accordance with the present invention, from commercial sources such as Ciphergen Biosystems (Fremont, CA), Perkin Elmer (Packard BioScience Company (Meriden CT), Zyomyx (Hayward, CA), and Phylos (Lexington, MA). Exemplary of these biochips are those described in U.S. patents No. 6,225,047, supra, and No. 6,329,209 (Wagner et al.), and in PCT publications WO 99/51773 (Kuimelis and Wagner) and WO 00/56934 (Englert *et al.*).

[0077] More specifically, biochips produced by Ciphergen Biosystems have surfaces, presented on an aluminum substrate in strip form, to which are attached, at addressable locations, chromatographic or biospecific adsorbents. The surface of the strip is coated with silicon dioxide.

[0078] Illustrative of Ciphergen ProteinChip® arrays are biochips H4, SAX-2, WCX-2, and IMAC-3, which include a functionalized, cross-linked polymer in the

form of a hydrogel, physically attached to the surface of the biochip or covalently attached through a silane to the surface of the biochip. The H4 biochip has isopropyl functionalities for hydrophobic binding. The SAX-2 biochip has quaternary ammonium functionalities for anion exchange. The WCX-2 biochip has carboxylate functionalities for cation exchange. The IMAC-3 biochip has nitriloacetic acid functionalities that adsorb transition metal ions, such as Cu++ and Ni++, by chelation. These immobilized metal ions, in turn, allow for adsorption of biomarkers by coordinate covalent bonding. Thus, Ciphergen's IMAC ProteinChip® arrays are sold with reactive moieties that become adsorbent upon the addition by the user of a metal solution.

[0079] In keeping with the above-described principles, a substrate with an adsorbent is contacted with the sample, containing serum, for a period of time sufficient to allow biomarker that may be present to bind to the adsorbent. In one embodiment of the invention, more than one type of substrate with adsorbent thereon is contacted with the biological sample. For example, a sample may be applied to both a WCX and an IMAC chip. This technique can allow for even more definitive assessment of cancer status. After the incubation period, the substrate is washed to remove unbound material. Any suitable washing solutions can be used; preferably, aqueous solutions are employed.

[0080] An energy absorbing molecule then is applied to the substrate with the bound biomarkers. As noted, an energy absorbing molecule is a molecule that absorbs energy from an energy source such as a laser, thereby assisting in desorption of biomarkers from the substrate. Exemplary energy absorbing molecules include, as noted above, cinnamic acid derivatives, sinapinic acid and dihydroxybenzoic acid. Preferably sinapinic acid is used.

[0081] The biomarkers bound to the substrates are detected in a gas phase ion spectrometer such as a time-of-flight mass spectrometer. The biomarkers are ionized by an ionization source such as a laser, the generated ions are collected by an ion optic assembly, and then a mass analyzer disperses and analyzes the passing ions. The detector then translates information of the detected ions into mass-to-charge

ratios. Detection of a biomarker typically will involve detection of signal intensity. Thus, both the quantity and mass of the biomarker can be determined.

[0082] Data generated by desorption and detection of biomarkers can be analyzed with the use of a programmable digital computer. The computer program analyzes the data to indicate the number of markers detected, and optionally the strength of the signal and the determined molecular mass for each biomarker detected. Data analysis can include steps of determining signal strength of a biomarker and removing data deviating from a predetermined statistical distribution. For example, the observed peaks can be normalized, by calculating the height of each peak relative to some reference. The reference can be background noise generated by the instrument and chemicals such as the energy absorbing molecule which is set as zero in the scale.

[0083] The computer can transform the resulting data into various formats for display. The standard spectrum can be displayed, but in one useful format only the peak height and mass information are retained from the spectrum view, yielding a cleaner image and enabling biomarkers with nearly identical molecular weights to be more easily seen. In another useful format, two or more spectra are compared, conveniently highlighting unique biomarkers and biomarkers that are up- or down-regulated between samples. Using any of these formats, one can readily determine whether a particular biomarker is present in a sample.

[0084] Software used to analyze the data can include code that applies an algorithm to the analysis of the signal to determine whether the signal represents a peak in a signal that corresponds to a biomarker according to the present invention. The software also can subject the data regarding observed biomarker peaks to classification tree or ANN analysis, to determine whether a biomarker peak or combination of biomarker peaks is present that indicates lung cancer status. Analysis of the data may be "keyed" to a variety of parameters that are obtained either directly or indirectly from the mass spectrometric analysis of the sample. These parameters include, but are not limited to, the presence or absence of one or more peaks, the height of one or more peaks, the log of the height of one or more peaks, and other arithmetic manipulations of peak height data.

[0085] In another aspect, the present invention provides kits for aiding in the diagnosis of lung cancer status, which kits are used to detect biomarkers according to the invention. The kits screen for the presence of biomarkers and combinations of biomarkers that are differentially present in samples from normal subjects and subjects with lung cancer.

[0086] In one embodiment, the kit comprises a substrate having an adsorbent thereon, wherein the adsorbent is suitable for binding a biomarker according to the invention, and a washing solution or instructions for making a washing solution, in which the combination of the adsorbent and the washing solution allows detection of the biomarker using gas phase ion spectrometry, e.g., mass spectrometry. The kit may include more than type of adsorbent, each present on a different substrate.

[0087] In another embodiment, a kit of the invention may include a first substrate, comprising an adsorbent thereon, and a second substrate onto which the first substrate is positioned to form a probe, which can be inserted into a gas phase ion spectrometer, e.g., a mass spectrometer. In another embodiment, an inventive kit may comprise a single substrate that can be inserted into the spectrometer.

[0088] In a further embodiment, such a kit can comprise instructions for suitable operational parameters in the form of a label or separate insert. For example, the instructions may inform a consumer how to collect the sample or how to wash the probe. In yet another embodiment the kit can comprise one or more containers with biomarker samples, to be used as standard(s) for calibration.

[0089] In a preferred embodiment, the detection of biomarkers for diagnosis of lung cancer in a subject entails contacting a sample from a subject or patient, preferably a serum sample, with a substrate having an adsorbent thereon under conditions that allow binding between the biomarker and the adsorbent, and then detecting the biomarker bound to the adsorbent by gas phase ion spectrometry, preferably by Surface Enhanced Laser Desorption/Ionization (SELDI) mass spectrometry. The biomarkers are ionized by an ionization source such as a laser. The generated ions are collected by an ion optic assembly and accelerated toward an ion detector. Ions that strike the detector generate an electric potential that is digitized by a high speed time-array recording device that digitally captures the analog signal. Ciphergen's

ProteinChip® system employs an analog-to-digital converter (ADC) to accomplish this. The ADC integrates detector output at regularly spaced time intervals into time-dependent bins. The time intervals typically are one to four nanoseconds long. Furthermore, the time-of-flight spectrum ultimately analyzed typically does not represent the signal from a single pulse of ionizing energy against a sample, but rather the sum of signals from a number of pulses. This reduces noise and increases dynamic range. This time-of-flight data is then subject to data processing. In Ciphergen's ProteinChip® software, data processing typically includes TOF-to-M/Z transformation, baseline subtraction, high frequency noise filtering. Thus, both the quantity and mass of the biomarker can be determined.

[0090] The detection of the biomarkers can be enhanced by using certain selectivity conditions, *e.g.*, adsorbents or washing solutions. In one embodiment, the same or similar selectivity conditions that were used to discover the biomarkers are used in the method of detecting the biomarker in the sample. For example, immobilized metal affinity capture chips such as the Cu(II) IMAC3 and weak cationic exchange chips such as the WCX2 chips are preferred as the adsorbents for biomarker detection. However, other adsorbents can be used, as long as they have the binding characteristics suitable for binding the biomarkers.

[0091] More particularly, armed with the information regarding the biomarkers identified herein, various methods can be used to recognize patterns of doublets, triplets, and higher combinations of biomarkers according to the invention. These methods take raw data regarding which peaks are present and their intensity and provide a differential diagnosis of lung cancer versus normal for a sample.

[0092] Thus, the process can be divided into the learning phase and the classification phase. In the learning phase, a learning algorithm is applied to a data set that includes members of the different classes that are meant to be classified, for example, data from a plurality of samples diagnosed as cancer and data from a plurality of samples assigned a negative diagnosis. The methods used to analyze the data include, but are not limited to, artificial neural network, support vector machines, genetic algorithm and self-organizing maps and classification and regression tree analysis. These methods are described, for example, in WO 01/31579, May 3, 2001

(Barnhill *et al.*); WO 02/06829, January 24, 2002 (Hitt *et al.*) and WO 02/42733, May 30, 2002 (Paulse *et al.*). The learning algorithm produces a classifying algorithm. The classifier is keyed to elements of the data, such as particular markers and particular intensities of markers, usually in combination, that can classify an unknown sample into one of the two classes. The classifier is ultimately used for diagnostic testing.

[0093] Software, both freeware and proprietary software, is readily available to analyze such patterns in data, and to devise additional patterns with any predetermined criteria for success. Those biomarkers which by themselves are predictive of a differential diagnosis of lung cancer versus normal do not require pattern recognition software to analyze the data.

[0094] The following examples are offered by way of illustration, and are not limiting.

Example I. Fractionation of serum

Buffers:

1. U9 (9M urea, 2% CHAPS, 50mM Tris-HCl pH9)
2. U1 (1M urea, 0.22% CHAPS, 50mM Tris-HCl pH9)
3. wash buffer 1: 50mM Tris-HCl with 0.1% n-octyl β-D-Glucopyranoside (OGP) pH9
4. wash buffer 2: 100mM sodium phosphate with 0.1% OGP pH7
5. wash buffer 3: 100mM sodium acetate with 0.1% OGP pH5
6. wash buffer 4: 100mM sodium acetate with 0.1% OGP pH4
7. wash buffer 5: 50mM sodium citrate with 0.1% OGP pH3
8. wash buffer 6: 33.3% isopropanol / 16.7% acetonitrile / 0.1% trifluoroacetic acid in water.

[0095] Thirty microliters of U9 buffer were added to 20μL of serum in a tube and were mixed at 4°C for 20 minutes. Ion exchange resin (Q Ceramic HyperDF ion exchange resin, Biosepra SA, France) was washed 3 times with 5 bed volumes of 50mM Tris-HCl pH9 and stored in 50% suspension. To each well of a 96-well filter plate (96-well Silent Screen filter plate, Loprodyne membrane, 0.45 micron pore,

Nalge Nunc International, USA), 125 μL of ion exchange resin (50% suspension) was added on a Biomek 2000 Automation Workstation (Beckman Coulter, Fullerton, CA), washed 3 times with 150μL U1 buffer, and vacuum dried. Urea-treated serum was transferred to each well of ion exchange resin. The serum tube was rinsed with 50μL of U1 buffer, which was also transferred to the corresponding well in filter plate. The filter plate was mixed on a platform shaker at 4°C for 30 minutes. Flow-through fraction was collected in a 96-well plate by vacuum suction (Fraction 1). Then, 100μL of wash buffer 1 was added to each well of filter plate and mixed for 10 minutes at room temperature. Eluant was collected into the same 96-well plate (Fraction 1). Resins in the filter plate were subsequently washed two times each with 100μL wash buffers 2, 3, 4, 5 and 6. Each eluant (total volume of 200μL) was collected in a 96-well plate (Fractions 2,3,4,5 and 6).

Example 2. SELDI analysis of fractionated serum

[0096] ProteinChip® Arrays were set up in 96-well bioprocessors. Buffer delivery and sample incubation were performed on a Biomek 2000 Automation Workstation. Each serum fraction was analyzed on IMAC3 (loaded with copper) and WCX2 ProteinChip® Arrays in duplicates. IMAC3 copper and WCX2 arrays (Ciphergen Biosystems Inc, Fremont, CA) were equilibrated two times with 150μL of binding buffer (100mM sodium phosphate + 0.5M NaCl pH7 for IMAC3, 100mM sodium acetate pH4 for WCX2). Each serum fraction was diluted in the corresponding binding buffer (1/5 dilution for IMAC3 and 1/10 dilution for WCX2) and 100μL was applied to each ProteinChip® array. Incubation was performed on a platform shaker at room temperature for 30 minutes. Each array was washed three times with 150μL of corresponding binding buffer and rinsed two times with water. ProteinChip® arrays were air-dried. Sinapinic acid matrix (prepared in 50% acetonitrile, 0.5% trifluoroacetic acid) was applied to each array. ProteinChip® arrays were read on a ProteinChip® PBSII Reader (Ciphergen Biosystems Inc.) A total of 253 laser shots were averaged for each array.

[0097] All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if

each individual publication or patent document were so individually denoted. By their citation of various references in this document Applicants do not admit that any particular reference is "prior art" to their invention.

What we claim is:

1. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a protein selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310,

wherein the biomarker is differentially present in samples of a subject with lung cancer and a normal subject that is free of lung cancer.

2. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, and IM-155,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, and WM-70.

3. The method according to claim 1, wherein the protein is selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, and IM-454,

or from a second group consisting

(ii) WM-61, WM-447, WM-446, WM-133, and WM-119.

4. The method according to claim 1, which uses a single biomarker selected from the group consisting of the WM-446 and WM-447.

5. A method for qualifying lung carcinoma risk in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

6. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected either from a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-38, IM-266 and IM-454, IM-266 and IM-522, IM- 266 and IM-544, IM-266 and IM-471, IM-474 and IM-151, IM-474 and IM-156, IM-474 and IM-544, IM-474 and IM-38, IM-522 and IM-507, IM-522 and IM-156, and IM-522 and IM-440;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, WM-447 and WM-118, WM-447 and WM-473, WM-19 and WM-59, WM-19 and WM-473, WM-19 and WM-369, WM-61 and WM-154, WM-61 and WM-369, WM-118 and WM-59 and WM-282 and WM-127.

7. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a pair of peaks selected from either a first group consisting of

(i) IM-266 and IM-474, IM-266 and IM-544, and IM-156 and IM-522;

or from a second group consisting of

(ii) WM-447 and WM-59, WM-447 and WM-19, and WM-19 and WM-59.

8. The method according to claim 5, wherein the pattern-recognition analysis is keyed to a triplet of peaks selected from

(i) IM-266, IM-454 and IM-474; and IM-266, IM-474 and IM-544;

or wherein the analysis is keyed to

(ii) WM-447, WM-19 and WM-473.

9. A kit for detecting and diagnosing lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

10. A kit according to claim 9, further comprising a washing solution or instructions for making a washing solution.

11. A kit according to claim 9, wherein the substrate is a SELDI probe that comprises either (i) functionalities that adsorb transition metal ions by chelation or (ii) functionalities that allow for cation exchange.

12. A method for qualifying lung adenocarcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

13. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

14. The method according to claim 12, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

15. A method for qualifying status of lung adenocarcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-

389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429.

16. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, and WM-120.

17. The method according to claim 15, wherein the protein is selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290.

18. A kit for detecting and diagnosing lung adenocarcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of biomarkers selected from the group consisting of WM-447, WM-652, WM-61, WM-446, WM-290, WM-363, WM-133, WM-341, WM-285, WM-366, WM-282, WM-362, WM-310, WM-292, WM-120, WM-134, WM-276, WM-428, WM-277, WM-20, WM-119, WM-340, WM-48, WM-389, WM-450, WM-47, WM-343, WM-17, WM-583, WM-70, WM-706, WM-346, WM-466, WM-646, WM-384, WM-336, WM-294, WM-339, WM-473, WM-369, WM-38, WM-283, WM-685, WM-66, WM-55, WM-650, WM-307, WM-278, WM-342, and WM-429, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

19. A kit according to claim 18, further comprising a washing solution or instructions for making a washing solution.

20. A kit according to claim 18, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

21. A method for qualifying squamous cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290,

WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

22. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

23. The method according to claim 21, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

24. A method for qualifying status of squamous cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128.

25. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, and WM-70.

26. The method according to claim 24, wherein the protein is selected from the group consisting of WM-447, WM-61, WM-277, WM-446, and WM-133.

27. A kit for detecting and diagnosing squamous cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-447, WM-61, WM-277, WM-446, WM-133, WM-134, WM-363, WM-362, WM-276, WM-706, WM-203, WM-466, WM-366, WM-65, WM-70, WM-341, WM-429, WM-347, WM-17, WM-47, WM-431, WM-62, WM-473, WM-384, WM-438, WM-652, WM-282, WM-389, WM-290, WM-278, WM-456, WM-673, WM-340, WM-55, WM-455, WM-645, WM-138, WM-420, WM-450, WM-369, WM-279, WM-342, WM-471, WM-674, WM-120, WM-20, WM-287, WM-83, WM-154, and WM-128, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

28. A kit according to claim 27, further comprising a washing solution or instructions for making a washing solution.

29. A kit according to claim 27, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

30. A method for qualifying small cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

31. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

32. The method according to claim 30, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

33. A method for qualifying status of small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279.

34. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, and WM-685.

35. The method according to claim 33, wherein the protein is selected from the group consisting of WM-70, WM-706, WM-369, WM-447, and WM-61.

36. A kit for detecting and diagnosing small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers selected from the group consisting of WM-70, WM-706, WM-369, WM-447, WM-61, WM-652, WM-282, WM-446, WM-456, WM-134, WM-203, WM-646, WM-455, WM-65, WM-685, WM-473, WM-343, WM-466, WM-341, WM-340, WM-363, WM-339, WM-457, WM-86, WM-506, WM-72, WM-287, WM-82, WM-528, WM-85, WM-73, WM-138, WM-384, WM-83, WM-450, WM-310, WM-277, WM-79, WM-207, WM-278, WM-290, WM-366, WM-472, WM-420, WM-147, WM-55, WM-669, WM-357, WM-429, and WM-279, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

37. A kit according to claim 36, further comprising a washing solution or instructions for making a washing solution.

38. A kit according to claim 36, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

39. A method for qualifying non-small cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

40. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

41. The method according to claim 39, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

42. A method for qualifying status of non-small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38.

43. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, and WM-456.

44. The method according to claim 42, wherein the protein is selected from the group consisting of WM-341, WM-342, WM-343, WM-48, and WM-340.

45. A kit for detecting and diagnosing non-small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-341, WM-342, WM-343, WM-48, WM-340, WM-346, WM-47, WM-339, WM-389, WM-669, WM-447, WM-652, WM-154, WM-587, WM-456, WM-450, WM-283, WM-207, WM-436, WM-384, WM-61, WM-167, WM-382, WM-285, WM-650, WM-203, WM-119, WM-282, WM-686, WM-383, WM-429, WM-11, WM-208, WM-451, WM-473, WM-220, WM-685, WM-338, WM-71, WM-266, WM-70, WM-545, WM-675, WM-446, WM-120, WM-267, WM-466, WM-347, WM-153, and WM-38, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

46. A kit according to claim 45, further comprising a washing solution or instructions for making a washing solution.

47. A kit according to claim 45, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

48. A method for qualifying large cell lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

49. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447.

50. The method according to claim 48, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

51. A method for qualifying status of large cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488.

52. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, and WM-447..

53. The method according to claim 51, wherein the protein is selected from the group consisting of WM-16, WM-26, WM-499, WM-134, and WM-647.

54. A kit for detecting and diagnosing large cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-

545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

55. A kit according to claim 50, further comprising a washing solution or instructions for making a washing solution.

56. A kit according to claim 50, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

57. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

58. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

59. The method according to claim 57, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

60. A method for distinguishing lung adenocarcinoma from squamous lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from the group

consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, WM-211, WM-362, WM-133, WM-414, WM-277, WM-141, WM-64, WM-135, WM-447, WM-383, WM-338, WM-63, WM-142, WM-446, WM-186, WM-111, WM-445, WM-455, WM-276, WM-444, WM-181, WM-35, WM-285, WM-456, WM-39, WM-82, WM-17, WM-203, WM-83, WM-412, WM-96, WM-74, WM-457, WM-431, WM-340, and WM-49.

61. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, WM-347, WM-134, WM-36, WM-108, WM-99, WM-151, WM-289, WM-363, WM-61, WM-117, and WM-211.

62. The method according to claim 60, wherein the protein is selected from the group consisting of WM-62, WM-415, WM-152, WM-385, and WM-347.

63. A kit for distinguishing lung adenocarcinoma from squamous lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-16, WM-26, WM-499, WM-134, WM-647, WM-277, WM-310, WM-363, WM-446, WM-221, WM-648, WM-657, WM-290, WM-328, WM-447, WM-684, WM-183, WM-190, WM-686, WM-397, WM-466, WM-20, WM-17, WM-545, WM-47, WM-191, WM-147, WM-480, WM-590, WM-218, WM-285, WM-652, WM-651, WM-366, WM-403, WM-418, WM-430, WM-456, WM-714, WM-646, WM-109, WM-302, WM-587, WM-375, WM-131, WM-706, WM-398, WM-309, WM-55, and WM-488, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

64. A kit according to claim 63, further comprising a washing solution or instructions for making a washing solution.

65. A kit according to claim 63, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

66. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject

for a level of a protein selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-26, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

67. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

68. The method according to claim 66, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, and WM-79.

69. A method for distinguishing lung adenocarcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, WM-455, WM-313, WM-456, WM-86, WM-70, WM-246, WM-360, WM-190, WM-418, WM-83, WM-257, WM-138, WM-47, WM-252, WM-282, WM-60, WM-68, WM-325, WM-402, WM-411, WM-405, WM-75, WM-417, WM-387, WM-26, WM-410, WM-420, WM-164, WM-67, WM-66, WM-391, WM-340, WM-428, WM-198, WM-312, and WM-152.

70. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79, WM-73, WM-64, WM-320, WM-419, WM-85, WM-82, WM-53, WM-412, WM-440, and WM-455.

71. The method according to claim 69, wherein the protein is selected from the group consisting of WM-457, WM-72, WM-369, WM-78, WM-79.

72. A kit for distinguishing lung adenocarcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

73. A kit according to claim 72, further comprising a washing solution or instructions for making a washing solution.

74. A kit according to claim 72, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

75. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprised of analyzing a biological sample from said subject for a level of a protein selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37.

76. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

77. The method according to claim 75, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

78. A method for distinguishing squamous cell lung carcinoma from small cell lung carcinoma in a subject, comprising

(A) providing a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, and

(B) extracting data from the spectrum and subjecting the data to pattern-recognition analysis that is keyed to at least one peak selected from either a first group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37.

79. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, and WM-447.

80. The method according to claim 78, wherein the protein is selected from the group consisting of WM-276, WM-277, WM-362, WM-257, and WM-363.

81. A kit for distinguishing squamous cell lung carcinoma from small cell lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers WM-276, WM-277, WM-362, WM-257, WM-363, WM-347, WM-53, WM-254, WM-17, WM-252, WM-431, WM-513, WM-446, WM-355, WM-447, WM-133, WM-245, WM-52, WM-96, WM-238, WM-243, WM-138, WM-62, WM-580, WM-134, WM-240, WM-256, WM-203, WM-111, WM-95, WM-247, WM-157, WM-242, WM-556, WM-63, WM-239, WM-234, WM-274, WM-370, WM-301, WM-449, WM-74, WM-261, WM-467, WM-237, WM-262, WM-295, WM-288, WM-384, and WM-37, and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

82. A kit according to claim 81, further comprising a washing solution or instructions for making a washing solution.

83. A kit according to claim 81, wherein the substrate is a SELDI probe that comprises functionalities that allow for cation exchange.

84. Software for qualifying lung carcinoma status in a subject, comprising an algorithm for analyzing data extracted from a spectrum generated by mass spectroscopic analysis of a biological sample taken from the subject, wherein said data relates to one or more biomarkers selected from either a first group consisting of

(i) IM-522, IM-273, IM-520, IM-519, IM-454, IM-507, IM-521, IM-148, IM-266, IM-537, IM-471, IM-510, IM-544, IM-474, IM-155, IM-157, IM-176, IM-445, IM-177, IM-440, IM-468, IM-438, IM-547, IM-359, IM-436, IM-106, IM-455, IM-444, IM-158, IM-265, IM-50, IM-159, IM-156, IM-439, IM-157, IM-508, IM-514, IM-478, IM-473, IM-360, IM-435, IM-150, IM-151, IM-110, IM-51, IM-163, IM-437, IM-546, IM-153, and IM-268,

or from a second group consisting of

(ii) WM-61, WM-447, WM-446, WM-133, WM-119, WM-278, WM-134, WM-363, WM-282, WM-362, WM-120, WM-290, WM-65, WM-277, WM-70, WM-369, WM-17, WM-473, WM-47, WM-203, WM-276, WM-279, WM-62, WM-366, WM-456, WM-428, WM-384, WM-287, WM-420, WM-292, WM-431, WM-455, WM-20, WM-340, WM-105, WM-389, WM-63, WM-354, WM-450, WM-466, WM-296, WM-343, WM-341, WM-339, WM-55, WM-66, WM-48, WM-38, WM-138, and WM-310.

85. Software according to claim 84, wherein said algorithm carries out a pattern-recognition analysis that is keyed to data relating to at least one of the biomarkers.

86. Software according to claim 85, wherein said algorithm comprises classification tree analysis that is keyed to data relating to at least one of the biomarkers.

87. Software according to claim 85, wherein said algorithm comprises artificial neural network analysis that is keyed to data relating to at least one of the biomarkers.

88. A method for qualifying lung carcinoma status in a subject, comprised of analyzing a biological sample from said subject for a diagnostic level of a biomarker that is serum amyloid A protein or a fragment thereof.

89. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

90. A method according to claim 89, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

91. A method according to claim 88, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

92. A method according to claim 88, for qualifying risk of lung adenocarcinoma.

93. A method according to claim 88, for qualifying risk of squamous cell lung carcinoma.

94. A method according to claim 88, for qualifying risk of small cell lung carcinoma.

95. A method according to claim 88, for qualifying risk of non-small cell lung carcinoma.

96. A method according to claim 88, for qualifying risk of large cell lung carcinoma.

97. A kit for detecting and diagnosing lung carcinoma, comprising

(A) an adsorbent attached to a substrate that retains one or more of the biomarkers that are serum amyloid A protein or a fragment thereof.

and

(B) instructions to detect the biomarker(s) by contacting a sample with the adsorbent and detecting the biomarker(s) retained by the adsorbent.

98. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 2803, 3168, 3277, 3552, 3897, 4300, 4490, 4655, 5927, 6874, 7776, 7941, 8152, 8952, 9233, 10300, 10866, or 10851 Daltons.

99. A kit according to claim 98, wherein said serum biomarker has an apparent molecular weight of about 3168, 3277, 3552, 3897, 4300, 4490, 4655, 7776, 7941, 8152, 8952, or 10851 Daltons.

100. A kit according to claim 97, wherein said serum biomarker has an apparent molecular weight of about 11.5 to 11.7 kD.

101. A kit according to claim 97, further comprising a washing solution or instructions for making a washing solution.

102. A kit according to claim 97, wherein the substrate is a SELDI probe.

FIGURE 1A

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-1	2011	A	IM-37	3893	A	IM-72	54026	A	IM-109	2882	B
IM-2	2030	A	IM-38	3960	A	IM-73	60170	A	IM-110	2967	B
IM-3	2069	A	IM-39	3972	A	IM-75	74372	A	IM-111	2977	B
IM-4	2128	A	IM-40	3984	A	IM-76	75545	A	IM-112	2994	B
IM-5	2146	A	IM-41	4066	A	IM-77	77543	A	IM-113	3031	B
IM-6	2186	A	IM-42	4178	A	IM-78	79507	A	IM-114	3048	B
IM-7	2232	A	IM-43	4287	A	IM-79	89854	A	IM-115	3148	B
IM-8	2277	A	IM-44	4297	A	IM-80	101831	A	IM-116	3166	B
IM-9	2295	A	IM-45	4309	A	IM-81	104301	A	IM-117	3283	B
IM-10	2318	A	IM-46	4484	A	IM-82	125160	A	IM-118	3308	B
IM-11	2411	A	IM-47	4649	A	IM-83	132976	A	IM-119	3332	B
IM-12	2434	A	IM-48	4798	A	IM-84	149099	A	IM-120	3432	B
IM-13	2467	A	IM-49	5104	A	IM-85	2016	B	IM-121	3450	B
IM-14	2482	A	IM-50	5918	A	IM-86	2029	B	IM-122	3561	B
IM-15	2498	A	IM-51	6122	A	IM-87	2144	B	IM-123	3615	B
IM-16	2565	A	IM-52	6192	A	IM-88	2130	B	IM-124	3714	B
IM-17	2574	A	IM-53	6452	A	IM-89	2168	B	IM-125	3730	B
IM-18	2586	A	IM-54	6660	A	IM-90	2184	B	IM-126	3834	B
IM-19	2605	A	IM-55	7766	A	IM-91	2200	B	IM-127	3899	B
IM-20	2722	A	IM-56	8145	A	IM-92	2284	B	IM-128	3969	B
IM-21	2746	A	IM-57	8954	A	IM-93	2299	B	IM-129	3986	B
IM-22	2788	A	IM-58	9312	A	IM-94	2314	B	IM-130	3997	B
IM-23	2855	A	IM-59	9449	A	IM-95	2414	B	IM-131	4013	B
IM-24	2871	A	IM-60	10272	A	IM-96	2428	B	IM-132	4181	B
IM-25	2984	A	IM-61	11663	A	IM-97	2451	B	IM-133	4297	B
IM-26	3030	A	IM-62	13376	A	IM-98	2466	B	IM-134	4311	B
IM-27	3144	A	IM-63	14698	A	IM-99	2483	B	IM-135	4465	B
IM-28	3243	A	IM-64	15190	A	IM-100	2565	B	IM-136	4484	B
IM-29	3273	A	IM-64	66758	A	IM-101	2583	B	IM-137	4579	B
IM-30	3290	A	IM-65	15951	A	IM-102	2597	B	IM-138	4608	B
IM-31	3369	A	IM-66	15172	A	IM-103	2697	B	IM-139	4669	B
IM-32	3445	A	IM-67	15925	A	IM-104	2715	B	IM-140	4747	B
IM-33	3483	A	IM-68	23436	A	IM-105	2740	B	IM-141	4862	B
IM-34	3676	A	IM-69	39794	A	IM-106	2752	B	IM-142	4891	B
IM-35	3779	A	IM-70	44166	A	IM-107	2767	B	IM-143	5033	B
IM-36	3793	A	IM-71	46890	A	IM-108	2865	B	IM-144	5077	B

FIGURE 1B

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-145	5099	B	IM-181	16018	B	IM-217	2130	C	IM-253	3733	C
IM-146	5143	B	IM-182	17346	B	IM-218	2145	C	IM-254	3833	C
IM-147	5158	B	IM-183	18311	B	IM-219	2167	C	IM-255	3900	C
IM-148	5272	B	IM-184	22586	B	IM-220	2182	C	IM-256	4010	C
IM-149	5306	B	IM-185	23422	B	IM-221	2199	C	IM-257	4145	C
IM-150	5349	B	IM-186	27969	B	IM-222	2211	C	IM-258	4187	C
IM-151	5364	B	IM-187	33283	B	IM-223	2230	C	IM-259	4299	C
IM-152	5421	B	IM-188	39808	B	IM-224	2250	C	IM-260	4466	C
IM-153	5554	B	IM-189	43110	B	IM-225	2280	C	IM-261	4582	C
IM-154	5711	B	IM-190	44454	B	IM-226	2297	C	IM-262	4813	0
IM-155	5876	B	IM-191	47215	B	IM-227	2317	C	IM-263	4876	C
IM-156	5916	B	IM-192	53784	B	IM-228	2412	C	IM-264	5032	C
IM-157	5931	B	IM-193	55952	B	IM-229	2428	C	IM-265	5347	C
IM-158	5988	B	IM-194	60573	B	IM-230	2468	C	IM-266	5365	C
IM-159	6137	B	IM-195	66346	B	IM-231	2481	C	IM-267	5932	C
IM-160	6200	B	IM-196	73387	B	IM-232	2498	C	IM-268	7767	C
IM-161	6443	B	IM-197	79203	B	IM-233	2567	C	IM-269	7973	C
IM-162	6644	B	IM-198	89302	B	IM-234	2585	C	IM-270	8143	C
IM-163	6958	B	IM-199	94226	B	IM-235	2599	C	IM-271	9187	C
IM-164	7481	B	IM-200	99358	B	IM-236	2698	C	IM-272	9293	C
IM-165	7568	B	IM-201	102096	B	IM-237	2715	C	IM-273	11705	C
IM-166	7765	B	IM-202	107199	B	IM-238	2745	C	IM-274	14041	C
IM-167	7955	B	IM-203	116936	B	IM-239	2766	C	IM-275	15114	C
IM-168	8144	B	IM-204	119487	B	IM-240	2867	C	IM-276	15939	C
IM-169	8698	B	IM-205	122103	B	IM-241	2885	C	IM-277	22321	C
IM-170	8821	B	IM-206	125431	B	IM-242	2998	C	IM-278	28001	C
IM-171	8944	B	IM-207	132052	B	IM-243	3052	C	IM-279	33296	C
IM-172	9138	B	IM-208	138518	B	IM-244	3096	C	IM-280	39770	C
IM-173	9298	B	IM-209	145147	B	IM-245	3151	C	IM-281	44460	C
IM-174	9390	B	IM-210	157502	B	IM-246	3167	C	IM-282	47307	C
IM-175	9516	B	IM-211	168579	B	IM-247	3286	C	IM-283	50625	C
IM-176	11711	B	IM-212	173391	B	IM-248	3303	C	IM-284	55898	C
IM-177	11914	B	IM-213	2011	C	IM-249	3335	C	IM-285	60882	C
IM-178	14033	B	IM-214	2030	C	IM-250	3448	C	IM-286	66294	C
IM-179	15110	B	IM-215	2050	C	IM-251	3619	C	IM-287	78892	C
IM-180	15838	B	IM-216	2096	C	IM-252	3709	C	IM-288	83848	C

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-289	89081	C	IM-325	2565	D	IM-361	13857	D	IM-397	2082	E
IM-290	94147	C	IM-326	2582	D	IM-362	14056	D	IM-398	2128	E
IM-291	99324	C	IM-327	2597	D	IM-363	15108	D	IM-399	2148	E
IM-292	107163	C	IM-328	2716	D	IM-364	15844	D	IM-400	2170	E
IM-293	110350	C	IM-329	2747	D	IM-365	22243	D	IM-401	2187	E
IM-294	113339	C	IM-330	2767	D	IM-366	25465	D	IM-402	2206	E
IM-295	116291	C	IM-331	2866	D	IM-367	28022	D	IM-403	2232	E
IM-296	122769	C	IM-332	2882	D	IM-368	33272	D	IM-404	2250	E
IM-297	131908	C	IM-333	2994	D	IM-369	40149	D	IM-405	2279	E
IM-298	145248	C	IM-334	3032	D	IM-370	43113	D	IM-406	2296	E
IM-299	159252	C	IM-335	3050	D	IM-371	44219	D	IM-407	2314	E
IM-300	165164	C	IM-336	3148	D	IM-372	47196	D	IM-408	2354	E
IM-301	174928	C	IM-337	3164	D	IM-373	51062	D	IM-409	2394	E
IM-302	196003	C	IM-338	3278	D	IM-374	56082	D	IM-410	2413	E
IM-303	2007	D	IM-339	3334	D	IM-375	58239	D	IM-411	2436	E
IM-304	2016	D	IM-340	3385	D	IM-376	60285	D	IM-412	2457	E
IM-305	2030	D	IM-341	3432	D	IM-377	66148	D	IM-413	2466	E
IM-306	2052	D	IM-342	3451	D	IM-378	73668	D	IM-414	2499	E
IM-307	2099	D	IM-343	3617	D	IM-379	77433	D	IM-415	2566	E
IM-308	2130	D	IM-344	3701	D	IM-380	79986	D	IM-416	2583	E
IM-309	2144	D	IM-345	3725	D	IM-381	80844	D	IM-417	2612	E
IM-310	2154	D	IM-346	3833	D	IM-382	88962	D	IM-418	2662	E
IM-311	2166	D	IM-347	3899	D	IM-383	94399	D	IM-419	2723	E
IM-312	2184	D	IM-348	4008	D	IM-384	99419	D	IM-420	2738	E
IM-313	2204	D	IM-349	4157	D	IM-385	108395	D	IM-421	2750	E
IM-314	2231	D	IM-350	4297	D	IM-386	116433	D	IM-422	2849	E
IM-315	2252	D	IM-351	4580	D	IM-387	123337	D	IM-423	2867	E
IM-316	2275	D	IM-352	4805	D	IM-388	131977	D	IM-424	3036	E
IM-317	2299	D	IM-353	6946	D	IM-389	145658	D	IM-425	3147	E
IM-318	2316	D	IM-354	7053	D	IM-390	152603	D	IM-426	3281	E
IM-319	2412	D	IM-355	7767	D	IM-391	159524	D	IM-427	3319	E
IM-320	2435	D	IM-356	7954	D	IM-392	196072	D	IM-428	3445	E
IM-321	2466	D	IM-357	8139	D	IM-393	2010	E	IM-429	3693	E
IM-322	2480	D	IM-358	9292	D	IM-394	2029	E	IM-430	3731	E
IM-323	2499	D	IM-359	11671	D	IM-395	2050	E	IM-431	3818	E
IM-324	2518	D	IM-360	13727	D	IM-396	2068	E	IM-432	3885	E

FIGURE 1D

MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION	MARKER ID	MW	FRACTION
IM-433	4136	E	IM-469	86211	E	IM-505	4174	F	IM-541	95033	F
IM-434	4169	E	IM-470	89407	E	IM-506	4362	F	IM-542	100310	F
IM-435	4257	E	IM-471	100270	E	IM-507	4473	F	IM-543	116889	F
IM-436	4277	E	IM-472	109638	E	IM-508	4631	F	IM-544	132711	F
IM-437	4355	E	IM-473	117132	E	IM-509	4822	F	IM-545	147276	F
IM-438	4369	E	IM-474	132843	E	IM-510	5862	F	IM-546	160768	F
IM-439	4470	E	IM-475	147160	E	IM-511	6192	F			
IM-440	4486	E	IM-476	152199	E	IM-512	6941	F			
IM-441	4541	E	IM-477	166461	E	IM-513	7626	F			
IM-442	4634	E	IM-478	176635	E	IM-514	7772	F			
IM-443	4841	E	IM-479	2011	F	IM-515	7957	F			
IM-444	5862	E	IM-480	2030	F	IM-516	8150	F			
IM-445	5911	E	IM-481	2128	F	IM-517	8954	F			
IM-446	6649	E	IM-482	2149	F	IM-518	9300	F			
IM-447	6952	E	IM-483	2186	F	IM-519	11545	F			
IM-448	7769	E	IM-484	2207	F	IM-520	11717	F			
IM-449	8148	E	IM-485	2279	F	IM-521	13887	F			
IM-450	8260	E	IM-486	2299	F	IM-522	14073	F			
IM-451	8785	E	IM-487	2319	F	IM-523	15196	F			
IM-452	9301	E	IM-488	2412	F	IM-524	15903	F			
IM-453	10071	E	IM-489	2434	F	IM-525	22460	F			
IM-454	11721	E	IM-490	2467	F	IM-526	23135	F			
IM-455	13910	E	IM-491	2485	F	IM-527	28135	F			
IM-456	15919	E	IM-492	2582	F	IM-528	33577	F			
IM-457	22422	E	IM-493	2605	F	IM-529	39813	F			
IM-458	28233	E	IM-494	2697	F	IM-530	42344	F			
IM-459	33490	E	IM-495	2751	F	IM-531	43274	F			
IM-460	43121	E	IM-496	2865	F	IM-532	44345	F			
IM-461	44558	E	IM-497	3036	F	IM-533	51007	F			
IM-462	46694	E	IM-498	3151	F	IM-534	56318	F			
IM-463	50954	E	IM-499	3372	F	IM-535	60079	F			
IM-464	54478	E	IM-500	3440	F	IM-536	66690	F			
IM-465	60041	E	IM-501	3488	F	IM-537	75122	F			
IM-466	66652	E	IM-502	3717	F	IM-538	78429	F			
IM-467	75580	E	IM-503	3890	F	IM-539	81249	F			
IM-468	79463	E	IM-504	4155	F	IM-540	89384	F			

FIGURE 2

RANK	MW	MARKER ID	RANK	MW	MARKER ID
1	14073	IM-522	39	117132	IM-473
2	11705	IM-273	40	13727	IM-360
3	11717	IM-520	41	4257	IM-435
4	11545	IM-519	42	5349	IM-150
5	11721	IM-454	43	5364	IM-151
6	4473	IM-507	44	2967	IM-110
7	13887	IM-521	45	6122	IM-51
8	5272	IM-148	46	6958	IM-163
9	5365	IM-266	47	4355	IM-437
10	75122	IM-537	48	160768	IM-546
11	100270	IM-471	49	5554	IM-153
12	5862	IM-510	50	7767	IM-268
13	132711	IM-544			
14	132843	IM-474			
15	5876	IM-155			
16	5932	IM-157			
17	11711	IM-176			
18	5911	IM-445			
19	11914	IM-177			
20	4486	IM-440			
21	79463	IM-468			
22	4369	IM-438			
23	100310	IM-542			
24	11671	IM-359			
25	4277	IM-436			
26	2752	IM-106			
27	13910	IM-455			
28	5862	IM-444			
29	5988	IM-158			
30	5347	IM-265			
31	5918	IM-50			
32	6137	IM-159			
33	5916	IM-156			
34	4470	IM-439			
35	5931	IM-157			
36	4631	IM-508			
37	7772	IM-514			
38	176635	IM-478			

Figure 3A

Peak ID	Fraction	Normal vs Cancer	Adeno vs Normal	Squamous vs Normal	Small Cell vs Normal	Non-small Cell vs Normal	Large Cell vs Normal	Adeno vs Squamous	Adeno vs Small Cell	Squamous vs Small Cell
WM-1	A	2010	2011	2010	2011	2009	2011	2009	2010	2010
WM-2	A	2032	2030	2031		2032	2032	2031		
WM-3	A	2052					2052			
WM-4	A	2069	2069	2069	2067	2062	2068	2069	2069	2069
WM-5	A	2088	2087	2088	2085	2079	2088	2088	2088	2088
WM-6	A	2168		2168		2170	2165			2168
WM-7	A	2233					2233			
WM-8	A	2277	2275	2274	2278	2277	2275	2277	2277	2277
WM-9	A	2296	2298	2296	2298	2298	2296	2296	2296	2298
WM-10	A	2390				2397	2390			
WM-11	A	2499	2500	2500	2498	2501	2493	2501	2499	2500
WM-12	A	2582	2580	2580	2584	2581	2582	2581	2582	2581
WM-13	A	2723					2722		2723	
WM-14	A	2739	2740	2740	2741	2740	2739	2738	2740	2739
WM-15	A	2751	2752	2752	2753	2753	2752	2752	2752	2752
WM-16	A	2768	2767	2769	2769		2768	2768	2768	2769
WM-17	A	2788	2788	2788	2790	2788	2789	2788	2788	2788
WM-18	A	2865	2864	2864	2866	2863	2864	2865	2864	2866
WM-19	A	2932	2932	2932	2933	2931	2932	2932	2932	2931
WM-20	A	2954	2952	2953	2958		2952	2952	2954	2955
WM-21	A	3082	3082	3073	3083	3091	3084	3084	3084	
WM-22	A	3157	3156	3157	3160	3160	3160	3155	3158	3158
WM-23	A	3187	3190	3186	3186	3188	3188	3190	3191	3189
WM-24	A	3215	3217	3218	3218	3218	3218	3215	3213	3214
WM-25	A	3240		3240			3244	3238	3238	3239
WM-26	A	3264	3262	3262	3264	3266	3268	3263	3263	3264
WM-27	A	3277	3274	3273	3275			3277	3274	3277
WM-28	A	3290	3288	3287	3288	3289	3288	3289	3291	3290
WM-29	A	3316	3318	3317	3320	3314	3318	3317	3317	3317
WM-30	A	3369	3365	3369	3372	3389	3366	3369	3371	3371
WM-31	A	3440	3437	3438	3441	3442	3439	3440	3440	3440
WM-32	A	3524	3526	3526	3526	3534	3527	3524	3524	3524
WM-33	A	3683	3683	3682	3684	3683	3683	3682	3683	3683
WM-34	A	3774	3775	3773	3778		3774		3776	3775
WM-35	A	3815	3816	3815	3816	3817	3816	3814	3816	3817
WM-36	A	3892	3892	3890	3893	3891	3891	3892	3892	3892
WM-37	A	3957	3960	3957	3960	3957	3956	3959	3958	3957
WM-38	A	3971	3976	3972	3975	3973	3978	3972	3974	3972
WM-39	A	4067	4069	4064	4066	4067	4066	4068	4069	
WM-40	A	4174	4175	4173	4173		4177		4173	4176
WM-41	A	4283	4285	4283	4286	4283	4283	4283	4283	4283
WM-42	A	4303	4305	4301	4301	4305	4301	4303	4303	4304
WM-43	A	4478	4478	4479	4475	4479	4479	4476	4476	4478
WM-44	A	4654	4651	4654	4650	4651	4650	4653	4651	4654
WM-45	A	5020	5024	5020	5016	5023	5020			
WM-46	A	5445	5448	5442	5439	5443	5444			
WM-47	A	5911	5903	5911	5905	5911	5910	5913	5911	5913
WM-48	A	6122	6125	6125	6126	6127	6126	6116	6116	6115
WM-49	A	6439	6442	6444	6443	6442	6442	6443	6441	6444
WM-50	A	6637	6639	6639	6640	6639	6639	6640	6637	6639

Figure 3B

WM-51	A		6827		6839	6828	6831	6828	6829				
WM-52	A		7561		7584	7581	7556	7548	7649		7574		7576
WM-53	A		7758		7763	7755	7754	7754	7765		7769	7770	7770
WM-54	A		8141		8139	8136	8133	8137	8138		8141	8142	8143
WM-55	A		8611		8611	8608	8604	8605	8610		8615	8615	8615
WM-56	A		8944		8942	8945	8943	8945	8945		8945	8947	8946
WM-57	A		9142		9159	9143	9143	9148	9144		9142	9164	9162
WM-58	A		9303		9302	9300	9303	9301	9302		9298	9301	9301
WM-59	A		10074		10080	10078	10069	10077	10080			10076	
WM-60	A		10279		10270	10277	10276	10278	10276		10271	10269	10269
WM-61	A		10851		10657	10659	10852	10851	10844		10858	10852	10859
WM-62	A		11484		11490	11490	11496				11489	11490	11492
WM-63	A		11690		11699	11695	11704				11688	11687	11691
WM-64	A		12865		12677	12879	12882	12881	12878		12879	12880	12886
WM-65	A		13335			13324	13335				13330	13341	13338
WM-66	A		14698		14701	14700	14702	14703	14695		14731	14730	14702
WM-67	A		15142		15147	15143	15139	15153	15134		15144	15145	15142
WM-68	A		15881		15888	15890	15878	15891	15889		15892	15882	15889
WM-69	A		23472		23443	23452	23436	23449	23453		23455	23472	23458
WM-70	A		28117		28102	28100	28104	28093	28092		28129	28148	
WM-71	A		28767		28761	28758	28754	28753	28748				
WM-72	A		33374		33329	33380	33341	33373	33303		33399	33390	33406
WM-73	A		39833		39871	39882	39851	39803	39811		39848	39894	39830
WM-74	A		44439		44327	44464	44363	44293	44280		44556	44488	44552
WM-75	A		50167		49954	50098	49849				50125	49880	50037
WM-76	A		51358		51314	51317	51279	51284	51255		51348	51444	51175
WM-77	A		53798		53750	53866	53803	53710	53675		53924	53888	54027
WM-78	A		59230									59053	
WM-79	A		60086		60059	60010	59919	59913	60086		60103	60103	60398
WM-80	A		62328		62287	62109	62871	62239	62228			62560	62237
WM-81	A		63019		62929	62957		62990	62896		63049		63102
WM-82	A		66863		66655	67039	66698	68638	66650		66580	66670	66571
WM-83	A		68409		68432	68577	68356		68591		67923	68363	68533
WM-84	A		74706		74529	74662	74641	74515	74497		74663	74611	74675
WM-85	A		79108		79052	79081	79058	70020	79001		78978	79148	79224
WM-86	A		89320		88930	89516	89072	89198	89027		89358	89220	89554
WM-87	A		101107		100985	101117	100909	100864	100835		101168	101051	101195
WM-88	A		113053		112640	113102	112574	112081	112142		113000	112012	
WM-89	A		124396		124239	124241	124232	124547	124225		124317	124330	124620
WM-90	A		134813		134770	134468	134478	134111	134928		134748	134911	134561
WM-91	A		148016		147695	147924	147603	147592	147505		148122	147988	148177
WM-92	A		172245		171306	171726	171410	171675	171101		172342	172147	171899
WM-93	B		2011		2011	2013	2012	2012	2013		2010	2011	2012
WM-94	B		2067		2067						2068	2067	2066
WM-95	B		2081		2079	2079	2080	2079	2079		2082	2081	2080
WM-96	B		2229		2229	2228	2227	2228	2227		2229	2229	2228
WM-97	B		2278		2276	2276	2277	2278	2278		2276	2277	2278
WM-98	B		2294		2295	2294	2295	2297	2298		2294	2295	2295
WM-99	B		2429		2429	2429	2428	2428	2428		2429	2429	2425
WM-100	B		2500		2501	2500	2500	2501	2500		2498	2498	2497
WM-101	B		2580		2580	2580	2580	2580	2580		2581	2581	2581

Figure 3C

WM-102	B	2723	2722	2722	2722	2722	2722	2723	2723	2723
WM-103	B	2752	2753	2753	2748	2753	2753	2752	2746	2747
WM-104	B	2865	2864	2866	2865	2885	2884	2868	2865	2866
WM-105	B	2933	2933	2933	2934	2934	2934	2933	2933	2933
WM-106	B	3082	3082	3082	3082	3083	3082	3083	3082	3083
WM-107	B	3158	3157	3158	3158	3157	3158	3155	3155	3155
WM-108	B	3288	3287	3286	3287	3287	3287	3288	3286	3288
WM-109	B	3311	3310	3311	3310	3310	3310	3317		
WM-110	B	3445	3447	3447	3445	3446	3446		3445	3446
WM-111	B	3817	3816	3816	3818	3818	3816	3816	3818	3816
WM-112	B	3888	3890	3890	3889	3888	3888	3888	3889	3889
WM-113	B	4210	4216	4215	4210	4211	4210	4212	4210	4211
WM-114	B	4292	4291	4283	4292	4292	4291		4291	4290
WM-115	B	4416	4416	4417	4414	4414	4414			
WM-116	B	4596	4598	4600				4597	4597	4598
WM-117	B	4968	4988	4984	4968	4968	4968	4984	4965	4968
WM-118	B	5013	5008	5010	5012	5012	5012			
WM-119	B	5342	5348	5342	5842	5344	5345	5340	5344	5342
WM-120	B	5909	5915	5910	5911	5914	5914	5911	5910	5910
WM-121	B	6440	6445	6440	6438	6439	6442	6436	6439	6438
WM-122	B	6636	6843	6838	6835	6837	6637	6636	6836	6837
WM-123	B	6822	6846	6843	6822	6842	6844	6845	6828	6838
WM-124	B	7573		7570	7567			7579		7573
WM-125	B	7768	7772	7769	7769	7763	7758	7769	7769	7770
WM-126	B	7952	7940	7950	7940			7945	7938	7941
WM-127	B	8142	8149	8142	8136	8139	8138	8143	8146	8143
WM-128	B	8700	8703	8693	8701	8701	8700	8699	8698	8696
WM-129	B	8822	8826	8823	8820	8824	8822	8822	8824	8823
WM-130	B	8945	8952	8945	8940	8944	8941	8945	8944	8945
WM-131	B	9188	9191	9186	9187	9184	9185	9196	9194	9195
WM-132	B	9298	9303	9295	9297	9297	9298	9293	9205	9298
WM-133	B	11520	11515	11513		11517		11515	11521	11532
WM-134	B	11711	11709	11701	11710	11717	11716	11709	11709	11701
WM-135	B	14063	14069	14083	14084	14063	14059	14062	14083	14066
WM-136	B	15132	15143	15136	15135	15133	15133	15138	15143	15141
WM-137	B	15882	15884	15873	15861	15879	15875	15872	15879	15879
WM-138	B	17304	17323	17296	17328	17331	17329	17302	17335	17307
WM-139	B	22297	22374	22289	22294	22291	22292	22375	22387	22373
WM-140	B	23330	23413	23448	23357	23388	23390	23448	23357	23420
WM-141	B	28114	28114	28117	28112	28110	28115	28117	28113	28114
WM-142	B	33369	33384	33358	33354	33357	33354	33369	33376	33384
WM-143	B	37524	37508	37651	37589	37583	37588	37566	37509	37652
WM-144	B	39686	39750	39687	39806	39718	39816	39689	39674	39689
WM-145	B	44590	44632	44591	44571	44586	44585	44627	44687	44586
WM-146	B	47400	47432	47419	47403	47407	47401	47433	47351	47422
WM-147	B	51287	51339	51287	51306	51299	51306	51323	51310	51309
WM-148	B	56293	56279	56299	56334	56287	56294	56251	56293	56277
WM-149	B	59889	59884	59862	59872	59809	59809	59866	59884	59869
WM-150	B	66480	66469	66471	66480	66451	66464	66480	66489	66488
WM-151	B	73122	73083	73066	73171	73050	73072	73090	73149	73180
WM-152	B	75411	75408	75482	75353	75400	75470	75264	75269	75317

Figure 3D

WM-153	B		78775	78769	78791	78804	78904	78916	78783	78772	78803
WM-154	B		89233	89208	89436	89215	89189	89201	89531	89295	89585
WM-155	B		94657	94635	94624	94578	94681	94638	94634	94590	94583
WM-156	B		99922	99871	100030	99986	99962	99849	100069	100005	100082
WM-157	B		107011	107055	107058	107066	107031	107049	107052	107006	107069
WM-158	B		110637	110703	110858	110369	110549	110683	110657	110383	110601
WM-159	B		117723	117778	117880	117925		117804	117862	118033	117940
WM-160	B		132592	132580	132587	132591	132549	132566	132602	132608	132612
WM-161	B		144790	144519	144275	144694	144419	144429	144770	144753	145083
WM-162	B		158637	158266	158834	158780	158488	159132	158659	158020	158971
WM-163	B		175311	174576	174729	174603	174900	174974	175470	175520	175363
WM-164	B		197100	197048	197070	197425	197001	197322	197080	197100	197052
WM-165	C		2011	2010	2011	2011	2010	2010	2010	2011	2010
WM-166	C		2031	2032	2029	2032	2032	2032	2032	2032	2032
WM-167	C		2078	2078	2076	2077	2080	2077	2079	2078	2079
WM-168	C		2168	2168	2166	2166	2166	2167	2167	2168	
WM-169	C		2184	2183	2183	2183	2184	2184	2184	2183	2185
WM-170	C		2211	2211	2210	2212	2211	2211	2211	2211	2210
WM-171	C		2225	2228	2225	2226	2226	2225	2228	2228	2228
WM-172	C		2279	2278	2281	2275	2278	2277	2278	2277	2278
WM-173	C		2297	2296	2284	2302	2295	2298	2297	2297	2297
WM-174	C		2352	2354	2354	2355	2354	2353	2353	2353	2353
WM-175	C		2426	2428	2428	2431	2428	2427	2429	2427	2430
WM-176	C		2474	2479	2480	2487	2480	2479	2474	2480	2474
WM-177	C		2536	2539	2539	2542	2538	2538	2539	2538	2538
WM-178	C		2582	2580	2581	2585	2582	2581	2580	2580	2582
WM-179	C		2636	2636	2637	2639	2637	2635	2638	2636	2637
WM-180	C		2749	2750	2760	2757	2750	2750	2751	2750	2751
WM-181	C		2863	2864	2865	2864	2884	2883	2865	2884	2866
WM-182	C		2934	2934	2935	2933	2935	2934	2935	2934	2935
WM-183	C		3082	3083	3084	3080	3083	3081	3084	3082	3083
WM-184	C		3144			3144					
WM-185	C		3154	3151	3154	3158	3151	3151	3154	3153	3153
WM-186	C		3283	3283	3283	3280	3262	3284	3282	3281	3282
WM-187	C		3446	3448	3447	3460	3446	3443	3448	3441	3445
WM-188	C		3560	3555	3557	3565	3559	3559	3554	3556	3554
WM-189	C		3690	3686	3887	3686	3887	3885	3690	3685	3696
WM-190	C		3813	3817	3817	3813	3814	3814	3811	3814	3813
WM-191	C		3840	3839	3843	3844	3836	3839	3836	3842	3837
WM-192	C		3948	3951	3952	3954	3953				
WM-193	C		4141	4144	4132		4144	4134	4134	4141	4130
WM-194	C		4209	4213	4214	4204	4212	4209	4210	4210	4211
WM-195	C		4288	4289	4287		4291	4288	4285	4285	4267
WM-196	C		4408	4417	4413		4415	4413	4410	4410	4410
WM-197	C		4789	4794	4795		4793	4795	4789	4779	4801
WM-198	C		6637	6832	6632	6615	6830	6632	6627	6634	6832
WM-199	C		7771	7771	7774	7751	7767	7765	7774	7788	7775
WM-200	C		8141	8151	8151	8174	8149	8146	8151	8138	8152
WM-201	C		9185	9197	9199	9227	9187	9194	9204	9196	9195
WM-202	C		9300	9306	9303		9305	9300	9288	9298	9288
WM-203	C		11706		11704	11697	11685		11705	11701	11705

Figure 3E

WM-204	C	14093	14088	14080	14073	14056	14088	14086	14088	14069
WM-205	C	15148	15141	15146	15138	15139	15130	15139	15139	15139
WM-206	C	15882	15870	15868	15882	15867	15884	15867	15885	15868
WM-207	C	17395	17337	17387	17376	17369	17369	17337		
WM-208	C	22238	22250	22258	22250	22260	22252	22248	22251	22268
WM-209	C	28110	28117	28123	28129'	28128	28140	28110	28114	28129
WM-210	C	33310	33316	33323	33325	33320	33316	33314	33312	33339
WM-211	C	37250	37214	37276	37215	37278	37233	37236	37205	37270
WM-212	C	44511	44526	44513	44515	44444	44503	44542	44529	44518
WM-213	C	47360	47378	47424	47399	47403	47391	47384	47377	47423
WM-214	C	51210	51236	51290	51284	51297	51297	51237	51369	51288
WM-215	C	56204	56260	56240	56290	56176	56212	56298	56307	56336
WM-216	C	59781	59753	59764	59738	59695	59710	59758	59752	59769
WM-217	C	66438	66445	66448	66443	66391	66437	66463	66447	66462
WM-218	C	74748	74914	74685	74702	74791	74780	74860	74823	74587
WM-219	C	78816	78919	78889	78738	78835	78852	78909	78551	78846
WM-220	C	80232	80406	80450	80375	80360	80275	80557	80772	80458
WM-221	C	83685	83637	83699	83617	83687	83874	83506	83612	83436
WM-222	C	89017	88926	88891	88973	88888	88893	88914	88990	88960
WM-223	C	94672	94716	94702	94625	94624	94617	94725	94711	94736
WM-224	C	99819	99668	99713	99739	99685	99691	99738	99763	99786
WM-225	C	110774	110758	110664	110795	110925	110916	110629	110738	110815
WM-226	C	118513	118193	118238	118337	118243	117858	118217	118098	118344
WM-227	C	132423	132397	132440	132417	132434	132426	132417	132388	132435
WM-228	C	145134	145087	145761	145642	145400	145157	145477	145617	145594
WM-229	C	154912	154930	154787	154931	155086	154980	155075	155082	155075
WM-230	C	165497	165378	165442	165399	165420	165352	165431	165374	165437
WM-231	C	177303	177164	177380	177206	177355	177339	177058	177024	176677
WM-232	C	181213								
WM-233	C	196920	196888	197088	197005	196998	197049	196911	196871	197015
WM-234	D	2011	2010	2011	2010	2009	2009	2010	2010	2012
WM-235	D	2032	2032	2032	2032	2029	2030	2032	2031	2033
WM-236	D	2054	2054	2052	2053	2050	2052	2053	2053	2054
WM-237	D	2078	2079	2078	2080	2078	2077	2078	2078	2079
WM-238	D	2168	2167	2167	2167	2167	2167	2169	2168	2167
WM-239	D	2186		2185	2184	2185	2183		2185	2185
WM-240	D	2211	2212	2211	2212	2208	2210	2211	2211	2212
WM-241	D	2230	2230	2229	2231	2228	2229	2229	2229	2229
WM-242	D	2256	2256	2255	2256	2254	2254	2255	2255	2255
WM-243	D	2278	2277	2278	2277	2276	2278	2278	2278	2278
WM-244	D	2302	2299	2298	2300	2297	2298	2299	2299	2299
WM-245	D	2357	2354	2353	2354	2352	2354	2352	2353	2354
WM-246	D	2427	2428	2426	2428	2427	2427	2427	2428	2428
WM-247	D	2483	2481	2480	2483	2478	2479	2480	2481	2481
WM-248	D	2500	2499	2499	2500	2498	2500	2500	2500	2499
WM-249	D	2544	2538	2543	2531	2542	2543	2542		
WM-250	D	2578				2579	2581			
WM-251	D	2635	2637	2637	2637	2636	2637	2637	2637	2637
WM-252	D	2754	2751	2749	2754	2748	2749	2749	2750	2750
WM-253	D	2868	2865	2863	2867	2862	2863	2864	2884	2884
WM-254	D	2935	2937	2935	2938	2935	2938	2936	2936	2936

Figure 3F

WM-255	D	3081	3084	3082	3085	3080	3082	3083	3083	3083
WM-256	D	3153	3158	3157	3159	3155	3157	3154	3154	3153
WM-257	D	3281	3282	3282	3283	3285	3286	3283	3284	3282
WM-258	D	3376			3368				3370	3369
WM-259	D	3445	3447	3446	3443	3442	3444	3446	3446	3444
WM-260	D	3558	3559	3556	3558	3556	3557	3557	3559	3557
WM-261	D	3685	3688	3684	3688	3683	3684	3688	3688	3688
WM-262	D	3815	3819	3815	3816	3813	3814	3816	3816	3814
WM-263	D	3843	3845	3842	3843	3839	3839	3841	3842	3841
WM-264	D	3958	3957	3953	3953	3949	3950	3950	3951	3950
WM-265	D	4059	4052					4052	4051	
WM-266	D	4138	4132	4133	4131	4129	4133	4129	4129	4130
WM-267	D	4212	4210	4207	4202	4207	4209	4209	4208	4206
WM-268	D	4417								4410
WM-269	D	4794	4800	4795	4801	4798	4801	4797	4796	4795
WM-270	D	6436	6438	6437	6435	6432	6438	6437	6437	6437
WM-271	D	6646	6638	6629	6633	6632	6635	6637	6636	6630
WM-272	D	6959	6949	6948	6947	6941	6944	6951	6951	6948
WM-273	D	7573	7575	7574	7571			7574	7572	7573
WM-274	D	7776	7766	7760	7762	7762	7771	7767	7768	7784
WM-275	D	7944	7942	7943	7946			7944	7948	7947
WM-276	D	11505	11503	11496				11498	11500	11497
WM-277	D	11688	11671	11676	11673	11662	11676	11693	11693	11679
WM-278	D	13779	13777	13778	13777	13771	13776		13774	13773
WM-279	D	13889	13883	13886	13865	13871	13875	13888	13889	13892
WM-280	D	15142	15138	15136	15144	15126	15134	15136	15137	15140
WM-281	D	15879	15874	15885	15880	15863	15871	15891	15885	15888
WM-282	D	17347	17322	17345	17350	17341	17349	17323	17317	17346
WM-283	D	22275	22277	22305	22274	22288	22277	22282	22289	22297
WM-284	D	28121	28135	28137	28129	28130	28119	28138	28148	28135
WM-285	D	33372	33333	33384	33320	33347	33338	33334	33355	33367
WM-286	D	37251	37272	37381	37271		37306		37278	37232
WM-287	D	40291	40265	40259	40304	40255	40251	40274	40320	40260
WM-288	D	41131		41135	41133			41146	41162	41190
WM-289	D	44571	44513	44493	44549	44502	44519	44500	44558	44588
WM-290	D	51239	51234	51228	51224	51224	51209	51235	51236	51262
WM-291	D	59572	59648	59659	59685	59584	59614	59596	59655	59667
WM-292	D	66475	66435	66487	66404	66470	66460	66469	66446	66493
WM-293	D	73807	73556	73753	73787	73587	73587	73854	73775	73696
WM-294	D	75077	75088	75040	75064	75060	75133			
WM-295	D	78048		78096				78098	78043	78103
WM-296	D	80309	80410	80529	80224	80234	80226	80448	80761	80423
WM-297	D	81525		81643	81600			81559		81650
WM-298	D	88910	88834	88990	88849	88857	88875	88967	88840	89035
WM-299	D	94508	94507	94554	94451	94326	94400	94540	94514	94543
WM-300	D	99811	99766	99712	99806	99757	99752	99714	99820	99733
WM-301	D	107088	107205	107240	107177	107007	107003	107208	107188	107159
WM-302	D	110406	110412	110664	110341	110651	110654	110470	110341	110405
WM-303	D	117373	117342	117259	117365	117334	117458	117260	117416	117271
WM-304	D	132451	132436	132443	132454	132441	132436	132443	132451	132469
WM-305	D	145955	146222	145604	146253	145734	145982	145601	145763	145819

Figure 3G

WM-306	D	154336				153873	153876			
WM-307	D	160515	160431	160716	160431	160442	160441	160722	160347	160810
WM-308	D	165704	165342	165621	165737	165848	165672	165563	165189	165683
WM-309	D	175390	175807	175460	175478	174282	175447	175350	176446	175606
WM-310	D	182482	182180	182165	182438	182273	181723	182095	181799	181902
WM-311	E	2010	2011	2011	2010	2010	2010	2010	2014	2011
WM-312	E	2028	2029	2029	2029	2029	2029	2030	2031	2029
WM-313	E	2050	2052		2052		2051	2052	2052	2052
WM-314	E	2067	2067	2064	2068	2067	2067	2068	2066	2067
WM-315	E	2082	2081	2082	2081	2083	2081	2083	2081	2081
WM-316	E	2128		2127				2125		2125
WM-317	E	2165	2168	2168	2167	2168	2165	2166	2164	2167
WM-318	E	2183	2186	2185	2186	2186	2185	2181	2185	2181
WM-319	E	2212	2212	2212	2212	2211	2211	2212	2212	2212
WM-320	E	2231	2231	2232	2231	2231	2231	2232	2231	2233
WM-321	E	2276	2278	2279	2278	2278	2278	2276	2278	2279
WM-322	E	2295	2296	2294	2285	2298	2295	2295	2291	2295
WM-323	E	2311		2312				2311	2305	2311
WM-324	E	2389					2388			
WM-325	E	2482	2481	2481	2474	2481	2481	2481	2481	2481
WM-326	E	2500	2499	2499	2500	2498	2499	2499	2500	2500
WM-327	E	2588	2587	2567	2588	2567	2567	2587	2567	2587
WM-328	E	2582	2581	2580	2581	2580	2580	2581	2582	2581
WM-329	E	2737		2736	2738	2736	2736			
WM-330	E	2862	2888	2859	2861	2865	2886	2880	2865	2885
WM-331	E	3167	3158	3158	3159	3157	3158	3147	3148	3147
WM-332	E	3321	3318	3315	3319	3316	3316	3315	3316	3318
WM-333	E	3443	3442	3439	3445	3439	3439	3440	3439	3439
WM-334	E	3818	3817	3816	3818	3816	3816	3816	3817	3816
WM-335	E	3882	3891	3884		3894		3884		3886
WM-336	E	4054	4055	4054	4056	4055	4056	4055	4054	4053
WM-337	E	4146							4147	
WM-338	E	4211	4211	4210	4211	4205	4210	4212	4214	4208
WM-339	E	4256	4259	4254	4258	4259	4260	4257	4261	4256
WM-340	E	4355	4358	4363	4356	4356	4358	4357	4359	4353
WM-341	E	4474	4469	4466	4469	4469	4472	4469	4468	4468
WM-342	E	4543	4548	4539	4541	4540	4543	4540	4544	4537
WM-343	E	4628	4628	4617	4628	4624	4631	4628	4632	4625
WM-344	E	4829	4821	4817	4820					
WM-345	E	4956		4956				4956		4954
WM-346	E	5073	5070	5069	5070	5068		5069	5068	5066
WM-347	E	5846		5857		5846		5852	5864	5849
WM-348	E	6462		6461				6481	6461	
WM-349	E	6643	6643	6644	6643	6642	6642	6644	6645	6643
WM-350	E	6686	6679	6688	6681	6680	6677	6683	6684	6678
WM-351	E	6854	6886	6865	6866	6865	6865	6857	6847	6850
WM-352	E	6949		6945	6951	6951	6946	6940	6949	6951
WM-353	E	7193	7194	7198	7191	7191	7193			
WM-354	E	7773	7770	7768	7770	7769	7776	7772	7778	7769
WM-355	E	8148	8148	8145				8147	8147	8147
WM-356	E	8705	8701	8695	8703	8702	8704	8700	8700	8701

Figure 3H

WM-357	E	8828	8828	8834	8828	8827	8828	8831	8830	8830
WM-358	E	8955	8949		8955	8952	8953	8945	8957	8948
WM-359	E	9176	9174	9191	9176	9176	9178	9177		9194
WM-360	E	9300	9302	9297	9299	9301	9299	9301	9297	9298
WM-361	E	9470	9470	9478	9454	9452	9480	9470	9466	9477
WM-362	E	11530	11532	11515		11520		11533	11527	11527
WM-363	E	11714	11705	11699	11687	11687	11699	11714	11703	11716
WM-364	E	12462	12462	12450	12464	12452	12457	12461	12460	12458
WM-365	E	12822	12814	12804	12813	12822	12813	12817	12825	12818
WM-366	E	13897	13897	13876	13888	13898	13871	13901	13883	13887
WM-367	E	15151	15148	15150	15154	15154	15155	15155	15160	15154
WM-368	E	15891	15891	15891	15901	15899	15904	15899	15896	15896
WM-369	E	17354	17855	17370	17320	17381	17382	17363	17381	17385
WM-370	E	22359	22309	22345	22349	22322	22314	22364	22409	22332
WM-371	E	28169	28183	28161	28162	28185	28181	28183	28154	28172
WM-372	E	33429	33437	33451	33431	33426	33391	33425	33413	33428
WM-373	E	39455		39235						39238
WM-374	E	44637	44603	44598	44627	44591	44578	44608	44651	44675
WM-375	E	51376	51359	51378	51357	51429	51308	51507	51400	51409
WM-376	E	59798	59921	59842	59828	59847	59744	59920	59747	59799
WM-377	E	68557	68553	68538	68572	68568	68527	68561	68590	66604
WM-378	E	75365	75353	75469	75388	75201	75188	75468	75459	75413
WM-379	E	79354	79373	79334	79388	79836	79757	79390	79378	79327
WM-380	E	89411	89514	89288	89490	89370	89337	89421	89542	89361
WM-381	E	100001	100004	99991	99979	99952	100031	100148	100028	100001
WM-382	E	110157	110138	109854	109994	109816	109772	110474	110414	109921
WM-383	E	117446	117783	117603	117749	117739	117918	117699	117802	117485
WM-384	E	132840	132828	132821	132805	132827	132578	132657	132853	132842
WM-385	E	146553	146371	146507	146653	146206	146470	145812	146010	146853
WM-386	E	154171	152936					152549	153495	152534
WM-387	E	165715	165860	165723	165192	165727	165654	165692	165820	165575
WM-388	E	182679	182591	182624	182269	182299	182217	183093	182701	183356
WM-389	E	197326	197192	197348	197206	197254	197266	197249	197213	197301
WM-390	F	2011	2013	2010	2010	2012	2011	2011	2011	2011
WM-391	F	2031	2033	2029	2029	2030	2029	2029	2029	2029
WM-392	F	2049	2055		2049	2053	2054		2052	
WM-393	F	2066	2068	2068	2067	2071	2072	2070	2071	2067
WM-394	F	2085	2083	2080	2083	2084	2084	2084	2084	2080
WM-395	F	2126	2123	2126	2126	2125		2126	2125	2126
WM-396	F	2168	2164	2167	2167	2167	2165	2167	2166	2166
WM-397	F	2214	2216	2213	2214	2214	2212	2213	2213	2213
WM-398	F	2234	2232	2234	2233	2234	2233	2234	2233	2234
WM-399	F	2280	2267	2279	2277	2280	2278	2279	2278	2279
WM-400	F	2298	2298	2296	2297	2297	2297	2297	2298	2298
WM-401	F	2390	2386		2390	2389			2389	
WM-402	F	2413	2411	2413	2413	2412	2413	2413	2413	2412
WM-403	F	2482	2482	2482	2479	2474	2482	2482	2481	2482
WM-404	F	2501	2500	2500	2500	2501	2500	2501	2501	2500
WM-405	F	2569	2568	2569	2570	2569	2569	2568	2568	2566
WM-406	F	2582	2581	2582	2582	2581	2582	2581	2582	2582
WM-407	F	2697	2697	2697	2700	2695	2698		2696	

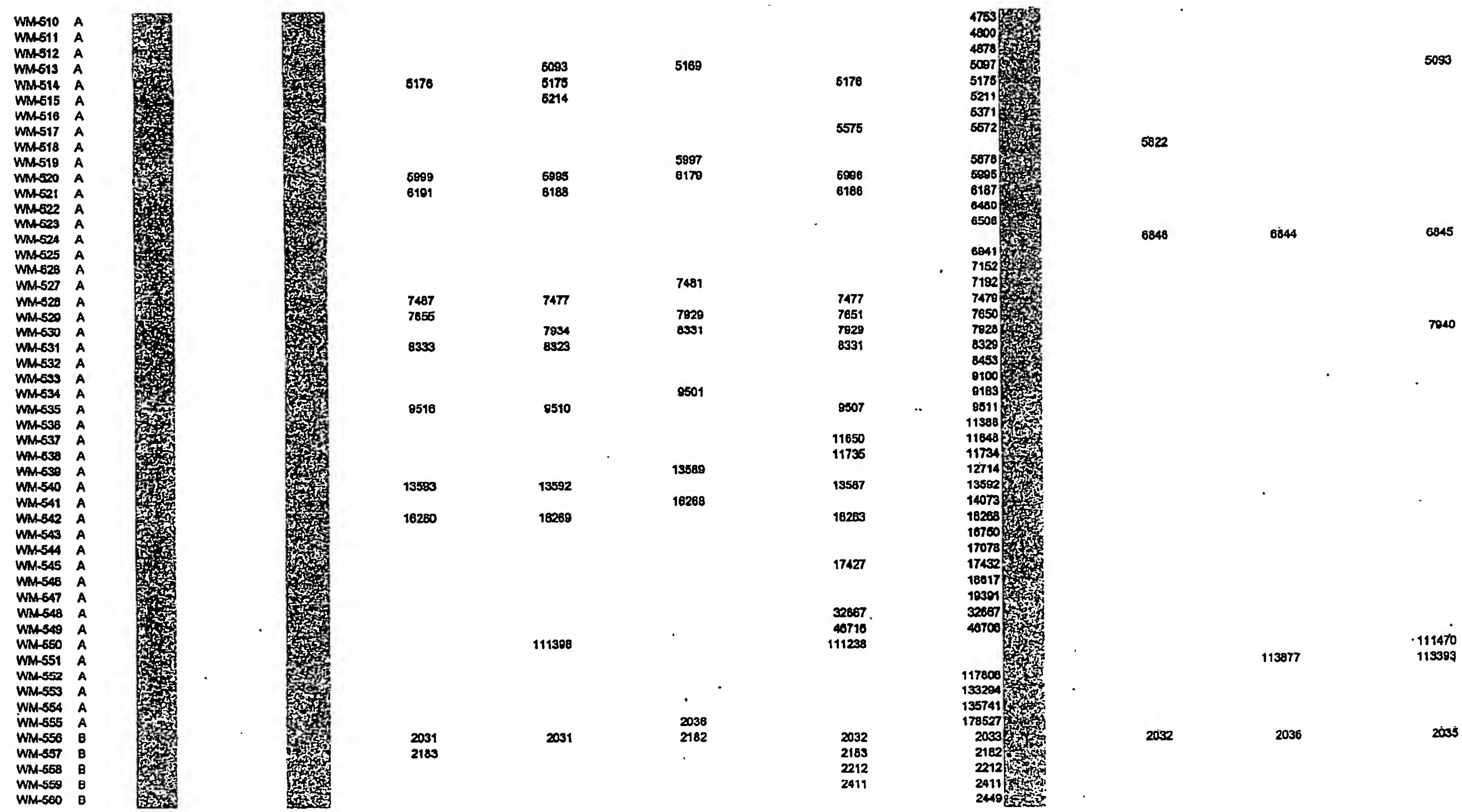
Figure 3I

WM-408	F	2723	2722	2723	2723	2723	2724	2723	2722	2722
WM-409	F	2753	2751	2751	2751	2750	2751	2751	2752	2750
WM-410	F	2866	2866	2867	2866	2885	2866	2865	2866	2866
WM-411	F	2980	2979	2981	2984	2979	2980	2979	2979	2979
WM-412	F	3151	3158	3159	3160	3156	3157	3152	3153	3152
WM-413	F	3373	3371	3372	3369	3372	3376	3371	3371	3372
WM-414	F	3441	3441	3441	3441	3443	3444	3439	3438	3439
WM-415	F	3487	3484	3487	3488	3487	3487	3486	3480	3492
WM-416	F	3580	3576		–		3579	3579	3580	3589
WM-417	F	3720	3714	3712	3721	3717	3719	3713	3717	3717
WM-418	F	3886	3886				3887		3886	
WM-419	F	4007							4001	
WM-420	F	4055	4057	4054	4054			4054	4055	4054
WM-421	F	4155	4154	4156	4158	4155	4166	4154	4155	4155
WM-422	F	4174	4179		4188					
WM-423	F	4201	4199	4203	4209	4205	4202	4204	4202	4212
WM-424	F	4215	4219					4216	4215	
WM-425	F	4358	4358						4361	
WM-426	F	4379	4375	4374	4367	4389	4370		4376	4376
WM-427	F	4414	4421	4414	4420	4416	4414	4420	4419	4417
WM-428	F	4473	4472	4479	4474	4475	4472	4473	4471	4473
WM-429	F	4836	4631	4630	4635	4835	4836	4630	4630	4828
WM-430	F	4832	4832	4831	4828	4824	4832	4837	4838	4831
WM-431	F	5868		5861	5860			5865	5863	5883
WM-432	F	6447	6448	6463	6459	6468	6484	6449	6451	6449
WM-433	F	6495	6491		6500			6494	6493	6498
WM-434	F	6662	6650	6848	6654	6658	6657	6650	6659	6848
WM-435	F	6849	6854	6847	6848	6840	6850	6848	6849	6847
WM-436	F	7191	7202	7191	7194	7192	7190	7185	7183	7182
WM-437	F	7601	7600		7609					
WM-438	F	7774	7774	7771	7777	7770	7771	7772	7771	7771
WM-439	F	7948		7949	7850			7951	7951	7949
WM-440	F	8238	8244	8239	8238	8244	8242	8241	8246	8231
WM-441	F	8710	8718	8708	8712	8711	8712	8706	8706	8701
WM-442	F	8836	8834	8831	8835	8837	8834	8831	8830	8831
WM-443	F	8980	8962	8955	8959	8961	8959	8958	8958	8955
WM-444	F	9175	9174	9171	9181	9176	9174	9171	9171	9171
WM-445	F	9486	9476	9468	9481	9483	9460	9476	9477	9467
WM-446	F	11583	11553	11568	11573	11562	11554	11565	11584	11565
WM-447	F	11743	11728	11737	11728	11717	11716	11738	11729	11736
WM-448	F	12473	12480	12458	12457	12458	12451	12461	12465	12484
WM-449	F	12819	12628	12625	12627	12828	12828		12622	12825
WM-450	F	13908	13918	13903	13907	13907	13903	13911	13908	13909
WM-451	F	14097	14086	14101	14086	14089	14095	14101	14098	14099
WM-452	F	15162	15172	15151	15162	15166	15150	15161	15159	15160
WM-453	F	15340		15341	15335			15344	15340	15337
WM-454	F	15889	15893	15890	15886	15888	15884		15914	15903
WM-455	F	17302	17313	17308	17323	17316	17324	17299	17299	17350
WM-456	F	17385	17390	17394	17402	17408	17404	17391	17390	
WM-457	F	23401	23275	23344	23332	23334	23283	23350	23395	23395
WM-458	F	28200	28215	28196	28260	28219	28179	28195	28190	28194

Figure 3J

WM-459	F	33594	33479	33451	33504	33468	33497	33487	33512	33520
WM-460	F	43440	43423	43430	43434	43393	43472	43547	43898	43415
WM-461	F	44487	44566	44523	44756	44668	44678	44679	44994	44892
WM-462	F	51132	51328	51176		51189	51293	51277	51226	51052
WM-463	F	56469	56487	56461	56410	56517	56506	56496	56610	56817
WM-464	F	59649	59575	59805	59844	59495	59726	59856	59452	59470
WM-465	F	66544	66615	66628	66608	66570	66532	66674	66587	66548
WM-466	F	75467	75421	75418	75561	75313	75298	75455	75464	75438
WM-467	F	78757		78306	78348	79194		78278	78346	78604
WM-468	F	83372			83331			83064	83307	83132
WM-469	F	89476	89779	89519	89751	89568	89549	89740	89776	89988
WM-470	F	94842	94791	94707	94826	94765	94742	94749	94628	94655
WM-471	F	100601	100418	100480	100614	100355	100398	100524	100594	100628
WM-472	F	116747	117035	116876	117130	116983	117127	116782	116803	116883
WM-473	F	132805	132795	133068	132640	132756	132729	132913	132707	132824
WM-474	F	148237	148470	148725	148960	148862	148862	148191	148500	148210
WM-475	F	160368	160334	161492		160793	160845	160004	160342	160570
WM-476	A					2131	2128			
WM-477	A				2184		2139			
WM-478	A				2414		2184			
WM-479	A						2413			
WM-480	A			2433			2434	2432	2432	2433
WM-481	A						2451			
WM-482	A				2568		2468			
WM-483	A		2567	2565		2567	2566			
WM-484	A						2599			
WM-485	A						2626			
WM-486	A						2637			
WM-487	A						2662			
WM-488	A			2873			2880			2876
WM-489	A					2732				
WM-490	A						2884			
WM-491	A				2978		2898			
WM-492	A		2981	2977		2982	2979			
WM-493	A						2994			
WM-494	A				3033		3015			
WM-495	A									
WM-496	A					3047	3044			
WM-497	A						3061			
WM-498	A						3398			
WM-499	A						3469			
WM-500	A						3490			
WM-501	A						3611			
WM-502	A						3720			
WM-503	A						4161			
WM-504	A						4191			
WM-505	A				4314	4214	4209			
WM-506	A									
WM-507	A		4526			4529	4526			
WM-508	A						4583			
WM-509	A						4726			

Figure 3k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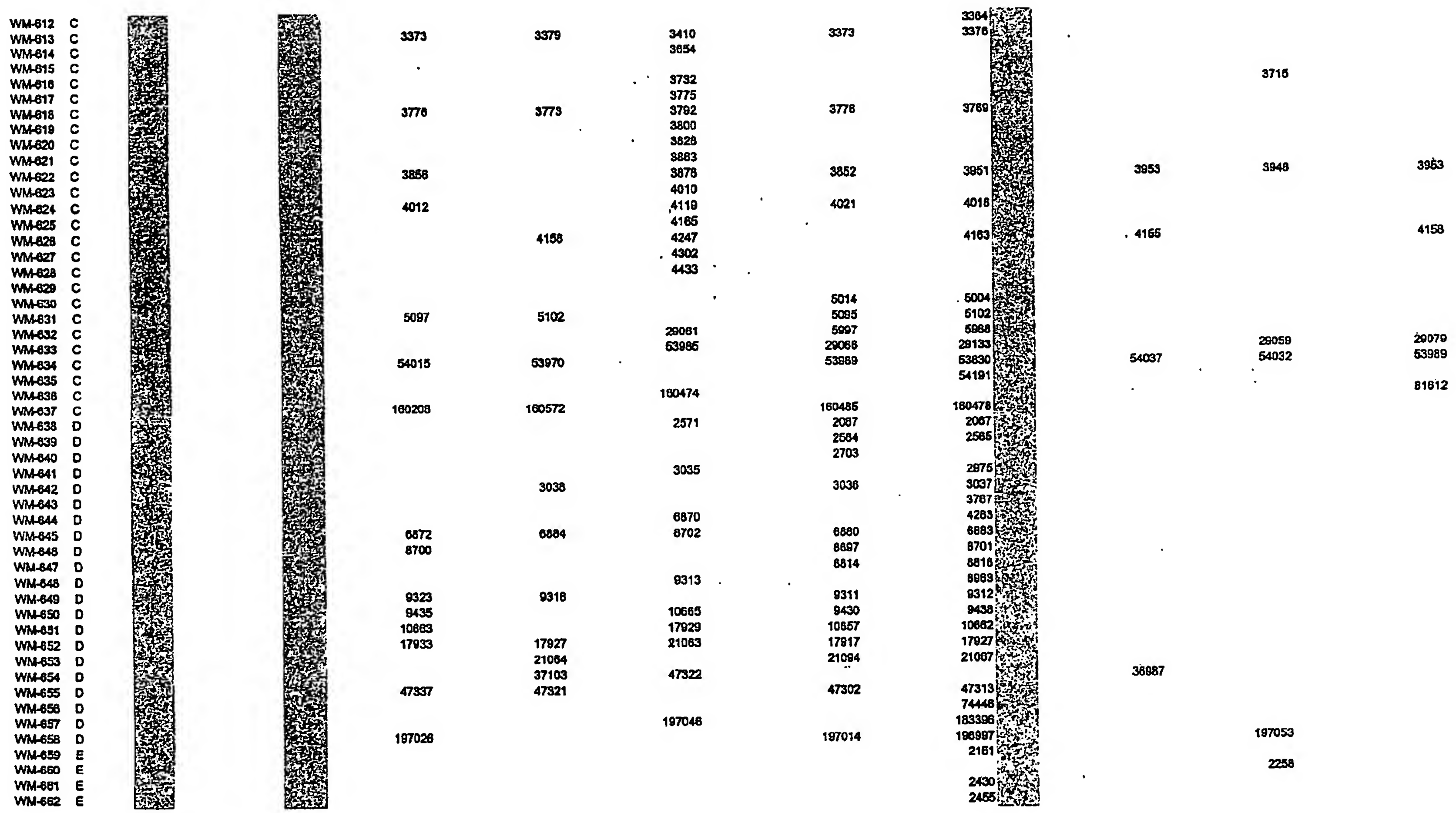
WM-513 A
WM-514 A
WM-515 A
WM-516 A
WM-517 A
WM-518 A
WM-519 A
WM-520 A
WM-521 A
WM-522 A
WM-523 A
WM-524 A
WM-525 A
WM-526 A
WM-527 A
WM-528 A
WM-529 A
WM-530 A
WM-531 A
WM-532 A
WM-533 A
WM-534 A
WM-535 A
WM-536 A
WM-537 A
WM-538 A
WM-539 A
WM-540 A
WM-541 A
WM-542 A
WM-543 A
WM-544 A
WM-545 A
WM-546 A
WM-547 A
WM-548 A
WM-549 A
WM-550 A
WM-551 A
WM-552 A
WM-553 A
WM-554 A
WM-555 A
WM-556 B
WM-557 B
WM-558 B
WM-559 B
WM-560 B
5176 5999 6191 7487 7655 8333 9516 13593 16280 2031 2183
5093 5175 5214 5995 6188 7477 7934 8323 9510 13592 16269 111398 2031
5169 5997 6179 7481 7929 8331 9501 13589 16268 2036 2182
5176 5575 5998 6186 7477 7651 7929 8331 9507 11650 11735 13587 16263 17427 32867 46716 111238 2032 2183 2212 2411
4753 4800 4878 5097 5175 5211 5371 5572 5878 5995 6187 6480 6506 6841 7152 7192 7479 7650 7928 8329 8453 9100 9183 9511 11388 11648 11734 12714 13592 14073 16268 16750 17078 17432 18617 19391 32867 46706 117806 133294 135741 178527 2033 2182 2212 2411 2449
5822 6848 2032
6844 113877 2036
5093 6845 7940 111470 113393 2035

Figure 3L

WM-561	B			2539	2467	2487			
WM-562	B	2540	2540		2539	2539			
WM-563	B			2978	2638	2639			
WM-564	B	2978	2978	3182	2976	2976			
WM-565	B	3181	3181	3274	3182	3182			
WM-566	B			3360	3274	3274			
WM-567	B	3380	3380	3377	3380	3380			
WM-568	B	3378	3376		3376	3376			
WM-569	B				3484	3485			
WM-570	B					3558			
WM-571	B			3771	3685	3685			
WM-572	B	3768	3772		3770	3770			
WM-573	B				3842	3843			
WM-574	B					3950			
WM-575	B			4091	4060	4060			
WM-576	B	4092	4091	4148	4092	4092		4092	
WM-577	B	4148	4143	4647	4147	4152			
WM-578	B	4647	4647	4792	4647	4647			
WM-579	B	4790	4784		4793	4792			
WM-580	B		4888	5443			4872		4877
WM-581	B	5442	5443	5988	5442	5442			
WM-582	B	5987	5990	6184	5986	5987			
WM-583	B	6187	6187	6605	6184	6184			
WM-584	B								
WM-585	B				10658	10663			
WM-586	B		16057				16085		
WM-587	B				17902	17807			41108
WM-588	B								
WM-589	B		53728	83865	53722	53727			
WM-590	B	83912	83843		83827	83853			
WM-591	B					156495			
WM-592	B					171918			
WM-593	C				2051	2053			
WM-594	C	2126	2126		2126	2126			
WM-595	C			2286		2255			
WM-596	C			2416					
WM-597	C			2458		2410			
WM-598	C	2458	2457			2462			
WM-599	C			2574		2495	2498	2498	
WM-600	C				2566	2568			
WM-601	C	2685		2728	2691	2679			
WM-602	C	2724		2840	2725	2723			
WM-603	C			2889					
WM-604	C			2982					
WM-605	C	2984	2980	3122	2983	2979			
WM-606	C								
WM-607	C	3163		3174	3162	3164			
WM-608	C	3178		3190	3177	3182			
WM-609	C			3252					
WM-610	C			3309					
WM-611	C								

Figure 3 M


WM-612 C
WM-613 C
WM-614 C
WM-615 C
WM-616 C
WM-617 C
WM-618 C
WM-619 C
WM-620 C
WM-621 C
WM-622 C
WM-623 C
WM-624 C
WM-625 C
WM-626 C
WM-627 C
WM-628 C
WM-629 C
WM-630 C
WM-631 C
WM-632 C
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3776
3858
4012
5097
54015
160208
6872
8700
9323
9435
10663
17933
47337
197026
3379
3773
4156
5102
53970
160572
3038
6884
9318
17927
21064
37103
47321
3410
3654
3732
3775
3792
3800
3828
3883
3878
4010
4119
4165
4247
4302
4433
29061
53985
160474
2571
3035
6870
8702
9313
10665
17929
21063
47322
197046
3373
3776
3852
4021
5014
5095
5997
29066
53989
160485
2087
2564
2703
3036
6880
8697
8814
9311
9430
10657
17917
21094
47302
197014
3364
3376
3769
3951
4016
4163
5004
5102
5988
28133
53830
54191
180478
2067
2565
2876
3037
3767
4283
6883
8701
8818
8963
9312
9438
10662
17927
21067
47313
74448
183396
196997
2161
2430
2455
3953
4155
54037
36987
3715
3948
29059
54032
197053
2258
3953
4158
29079
53989
81612

Figure 3 N

WM-663	E			2598	2468	2467			
WM-664	E	2599	2599		2596	2599			
WM-665	E	2730	2722		2723	2722	2729	2723	2723
WM-666	E	2751	2751		2751	2751	2751	2751	2751
WM-667	E			3375		3085			
WM-668	E		3378	4131		3378			3370
WM-669	E	4129	4129	4162	4130		4128		4125
WM-670	E	4162	4165		4162	4161			4165
WM-671	E		4267						
WM-672	E		4345						
WM-673	E		4485					4480	
WM-674	E		4835						
WM-675	E				4804	4803			
WM-676	E						4844	4849	4837
WM-677	E			6310	5988	5984			
WM-678	E			6445	6310	6310			
WM-679	E	6445		6484	6442	6444			6443
WM-680	E	6481			6482	6481			6482
WM-681	E								7944
WM-682	E					10942			
WM-683	E				13804				
WM-684	E			17919		14408			
WM-685	E	17923	17920	40286	17899	17903			
WM-686	E				40301	40292			
WM-687	E				78263				
WM-688	E		81987	84156					82092
WM-689	E	84031		94683	84188	84158			
WM-690	E	94747	94743		94734	94699			
WM-691	E								144070
WM-692	E						154782		154993
WM-693	E				178344	176742			
WM-694	F				2184	2183			
WM-695	F	2281		3322					
WM-696	F	3324	3319	4172	3314	3318			
WM-697	F	4167	4170		4174	4172	4170	4169	4169
WM-698	F			5010	4719	4715			
WM-699	F	5011	5009		5011	5004			
WM-700	F		6678				6679		6676
WM-701	F		7578				7581	7579	7580
WM-702	F		7624		7623	7821			
WM-703	F					8311			
WM-704	F				14425	14421			
WM-705	F			17943			15962		
WM-706	F	17935	17946		17945	17941			
WM-707	F								44156
WM-708	F	45416	45399	50956	46391	45385			
WM-709	F	50516		58844			50514	50434	
WM-710	F					58802			
WM-711	F								60463
WM-712	F						79936		
WM-713	F	82174	82044						

Figure 30.

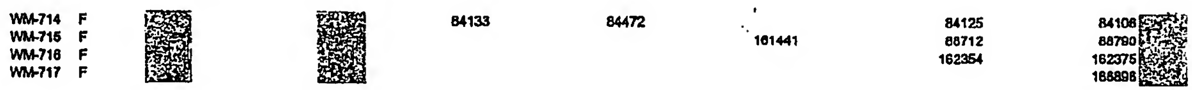
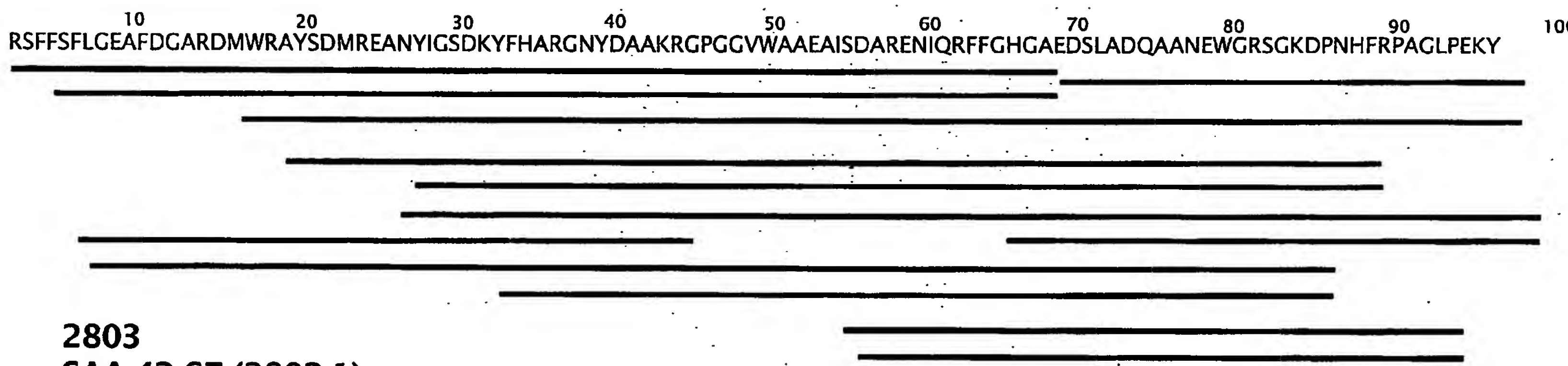

WM-714 F
WM-715 F
WM-716 F
WM-717 F
84133
84472
161441
84125
86712
162354
84106
86790
162375
166896

Figure 4A

Rank	Normal vs Cancer	Adeno vs Normal	Squamous vs Normal	Small Cell vs Normal	Non-small Cell vs Normal	Large Cell vs Normal	Adeno vs Squamous	Adeno vs Small Cell	Squamous vs Small Cell
1	WM-61	WM-447	WM-447	WM-70	WM-341	WM-16	WM-62	WM-457	WM-276
2	WM-447	WM-652	WM-61	WM-706	WM-342	WM-26	WM-415	WM-72	WM-277
3	WM-446	WM-61	WM-277	WM-369	WM-343	WM-499	WM-152	WM-369	WM-362
4	WM-133	WM-446	WM-446	WM-447	WM-48	WM-134	WM-385	WM-78	WM-257
5	WM-119	WM-290	WM-133	WM-61	WM-340	WM-647	WM-347	WM-79	WM-363
6	WM-278	WM-363	WM-134	WM-652	WM-346	WM-277	WM-134	WM-73	WM-347
7	WM-134	WM-133	WM-363	WM-282	WM-47	WM-310	WM-36	WM-64	WM-53
8	WM-363	WM-341	WM-362	WM-446	WM-339	WM-363	WM-108	WM-320	WM-254
9	WM-282	WM-285	WM-276	WM-456	WM-389	WM-446	WM-99	WM-419	WM-17
10	WM-362	WM-366	WM-706	WM-134	WM-669	WM-221	WM-151	WM-85	WM-252
11	WM-120	WM-282	WM-203	WM-203	WM-447	WM-648	WM-289	WM-82	WM-431
12	WM-290	WM-362	WM-468	WM-646	WM-652	WM-657	WM-363	WM-53	WM-513
13	WM-65	WM-310	WM-388	WM-455	WM-154	WM-290	WM-61	WM-412	WM-446
14	WM-277	WM-292	WM-65	WM-65	WM-587	WM-328	WM-117	WM-440	WM-355
15	WM-70	WM-120	WM-70	WM-685	WM-456	WM-447	WM-211	WM-455	WM-447
16	WM-369	WM-134	WM-341	WM-473	WM-450	WM-684	WM-362	WM-313	WM-133
17	WM-17	WM-276	WM-429	WM-343	WM-283	WM-183	WM-133	WM-458	WM-245
18	WM-473	WM-428	WM-347	WM-466	WM-207	WM-190	WM-414	WM-86	WM-52
19	WM-47	WM-277	WM-17	WM-341	WM-436	WM-686	WM-277	WM-70	WM-96
20	WM-203	WM-20	WM-47	WM-340	WM-384	WM-397	WM-141	WM-246	WM-238
21	WM-276	WM-119	WM-431	WM-363	WM-61	WM-466	WM-64	WM-360	WM-243
22	WM-279	WM-340	WM-62	WM-339	WM-167	WM-20	WM-135	WM-190	WM-138
23	WM-62	WM-48	WM-473	WM-457	WM-382	WM-17	WM-447	WM-418	WM-62
24	WM-366	WM-389	WM-384	WM-86	WM-285	WM-545	WM-383	WM-83	WM-580
25	WM-456	WM-450	WM-438	WM-506	WM-650	WM-47	WM-338	WM-257	WM-134
26	WM-428	WM-47	WM-652	WM-72	WM-203	WM-191	WM-63	WM-138	WM-240
27	WM-384	WM-343	WM-282	WM-287	WM-119	WM-147	WM-142	WM-47	WM-256
28	WM-287	WM-17	WM-389	WM-82	WM-282	WM-480	WM-446	WM-252	WM-203
29	WM-420	WM-583	WM-290	WM-528	WM-686	WM-590	WM-186	WM-282	WM-111
30	WM-292	WM-70	WM-278	WM-85	WM-383	WM-218	WM-111	WM-60	WM-95
31	WM-431	WM-706	WM-456	WM-73	WM-429	WM-285	WM-445	WM-68	WM-247
32	WM-455	WM-346	WM-673	WM-138	WM-11	WM-652	WM-455	WM-325	WM-157
33	WM-20	WM-466	WM-340	WM-384	WM-208	WM-651	WM-276	WM-402	WM-242
34	WM-340	WM-646	WM-55	WM-83	WM-451	WM-368	WM-444	WM-411	WM-556
35	WM-19	WM-384	WM-455	WM-460	WM-473	WM-403	WM-181	WM-405	WM-63
36	WM-389	WM-338	WM-645	WM-310	WM-220	WM-418	WM-35	WM-75	WM-239
37	WM-63	WM-294	WM-138	WM-277	WM-685	WM-430	WM-285	WM-417	WM-234
38	WM-438	WM-339	WM-420	WM-79	WM-338	WM-456	WM-456	WM-387	WM-274

Figure 4B

39	WM-450	WM-473	WM-450	WM-207	WM-71	WM-714	WM-39	WM-26	WM-370
40	WM-466	WM-369	WM-369	WM-278	WM-266	WM-646	WM-82	WM-410	WM-301
41	WM-296	WM-38	WM-279	WM-290	WM-70	WM-109	WM-17	WM-420	WM-449
42	WM-343	WM-283	WM-342	WM-366	WM-545	WM-302	WM-203	WM-164	WM-74
43	WM-341	WM-685	WM-471	WM-472	WM-675	WM-587	WM-83	WM-67	WM-261
44	WM-339	WM-66	WM-674	WM-420	WM-446	WM-375	WM-412	WM-66	WM-467
45	WM-55	WM-55	WM-120	WM-147	WM-120	WM-131	WM-96	WM-391	WM-237
46	WM-66	WM-650	WM-20	WM-56	WM-267	WM-706	WM-74	WM-340	WM-262
47	WM-48	WM-307	WM-287	WM-669	WM-466	WM-398	WM-457	WM-428	WM-295
48	WM-38	WM-278	WM-83	WM-357	WM-347	WM-309	WM-431	WM-198	WM-288
49	WM-138	WM-342	WM-154	WM-429	WM-153	WM-55	WM-340	WM-312	WM-384
50	WM-310	WM-429	WM-128	WM-279	WM-38	WM-488	WM-49	WM-152	WM-37



2803
SAA 42-67 (2802.1)

3168
SAA 69-97 (3167.3)

3277
SAA 39-68 (3276.6)

3552
SAA 38-70 (3552)

3897
SAA 64-98 (3897.2)

4300
SAA 54-93 (4302.5)

4490
SAA 53-93 (4489)

4655
SAA 5-44 (4655.0)

5927
SAA 32-<u>85</u> (5925.3)

6874
SAA 26-88 (6873.3)

7776
SAA 1-68 (7774.6)

7941
SAA 18-88 (7939.5)

8152
SAA 25-98 (8150)

8952
SAA 6-<u>85</u> (8950)

9233
SAA 16-*97* (9235)

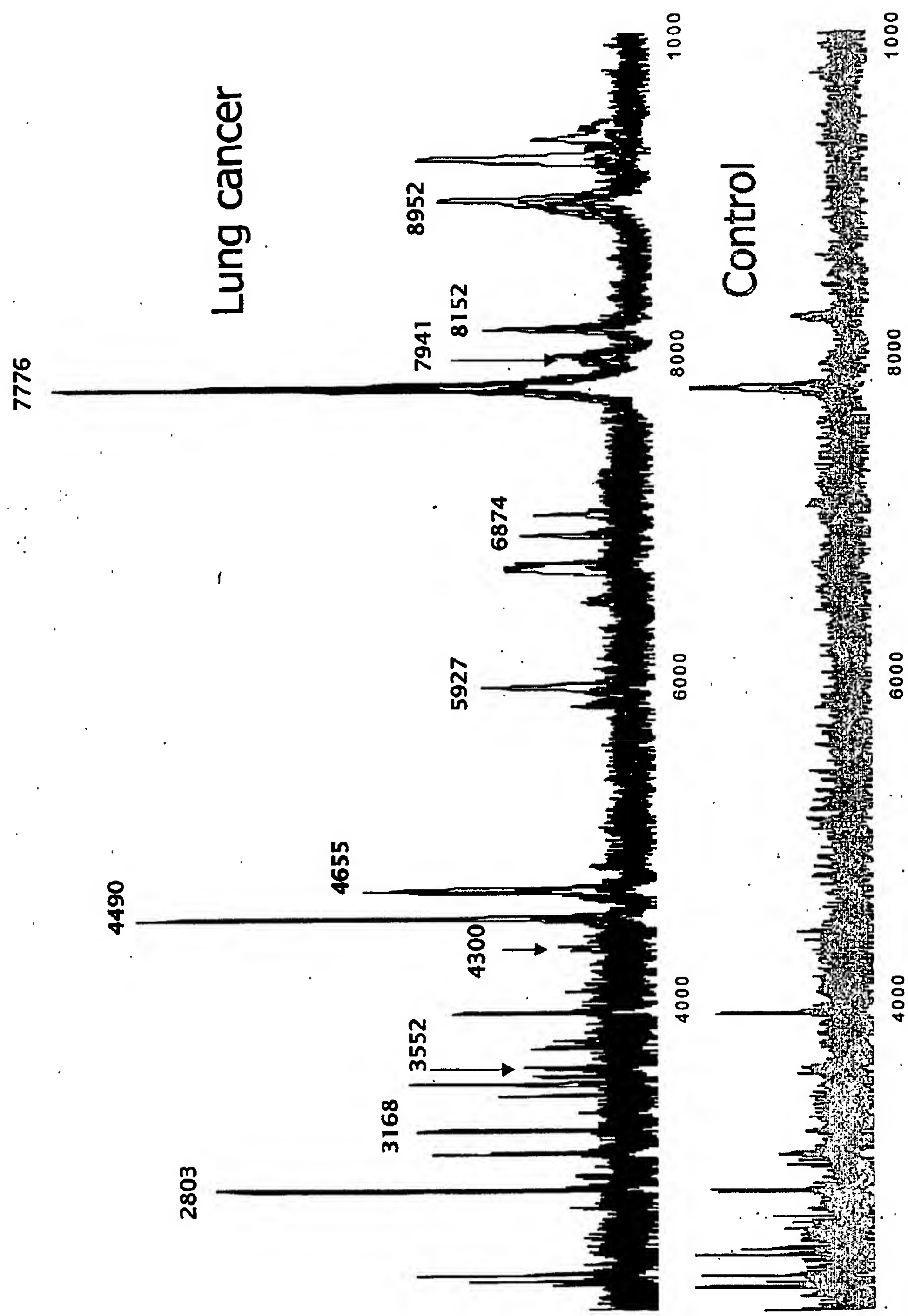
10300
SAA 6-*97* (10299.1)

10866
SAA 4-101 (10871.8)

10851
SAA 5-102 (10853.7)

Figure 5

Figure 6


Lung cancer
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7941
6874
5927
4655
4490
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3552
3168
2803
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8000
6000
4000
Control
1000
8000
6000
4000

FIGURE 7
Protein Profile of Selected Samples Q Fraction 1 WCX2

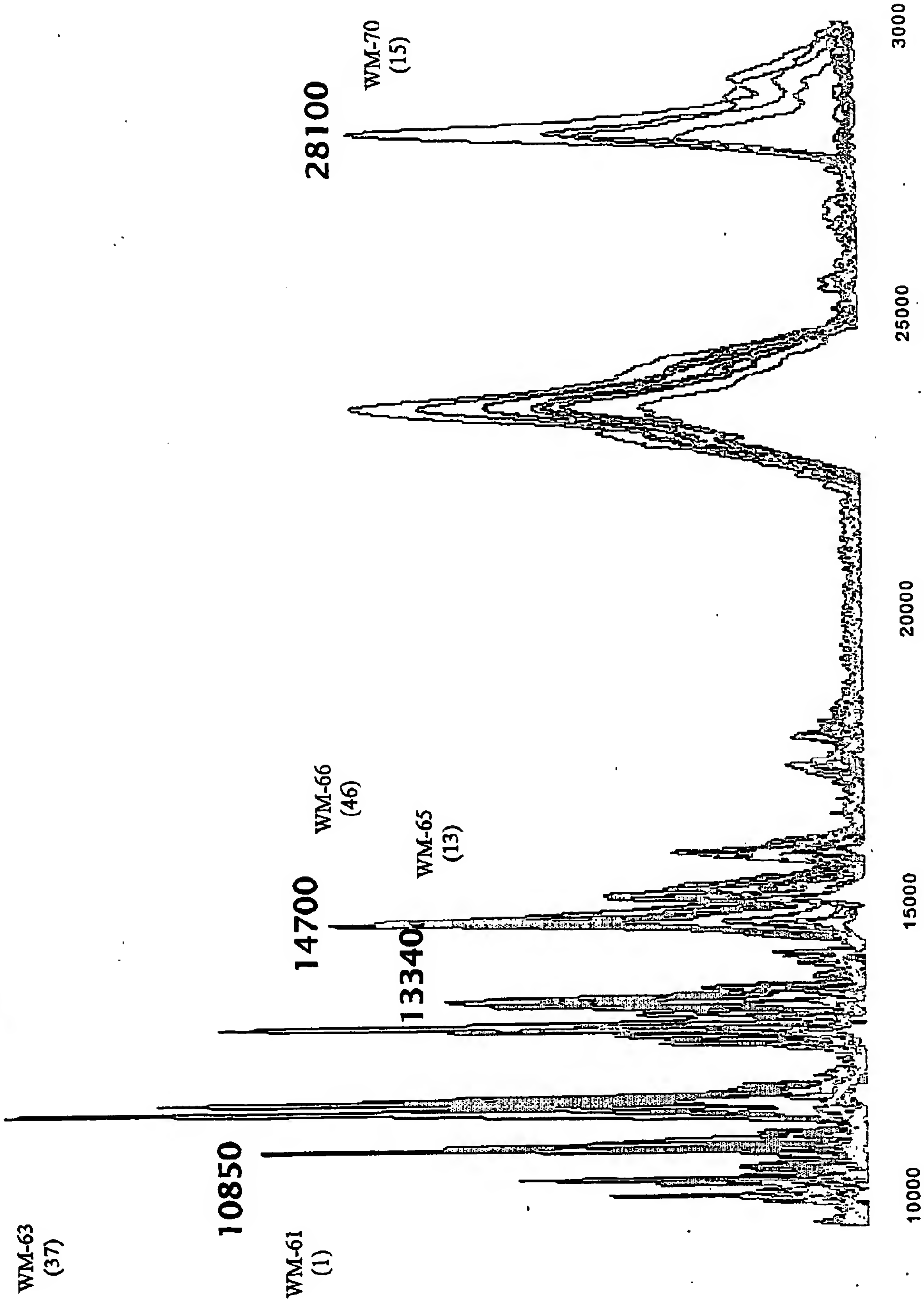


Figure 8
Protein Profile of Selected Samples Q Fraction 1 WCX2

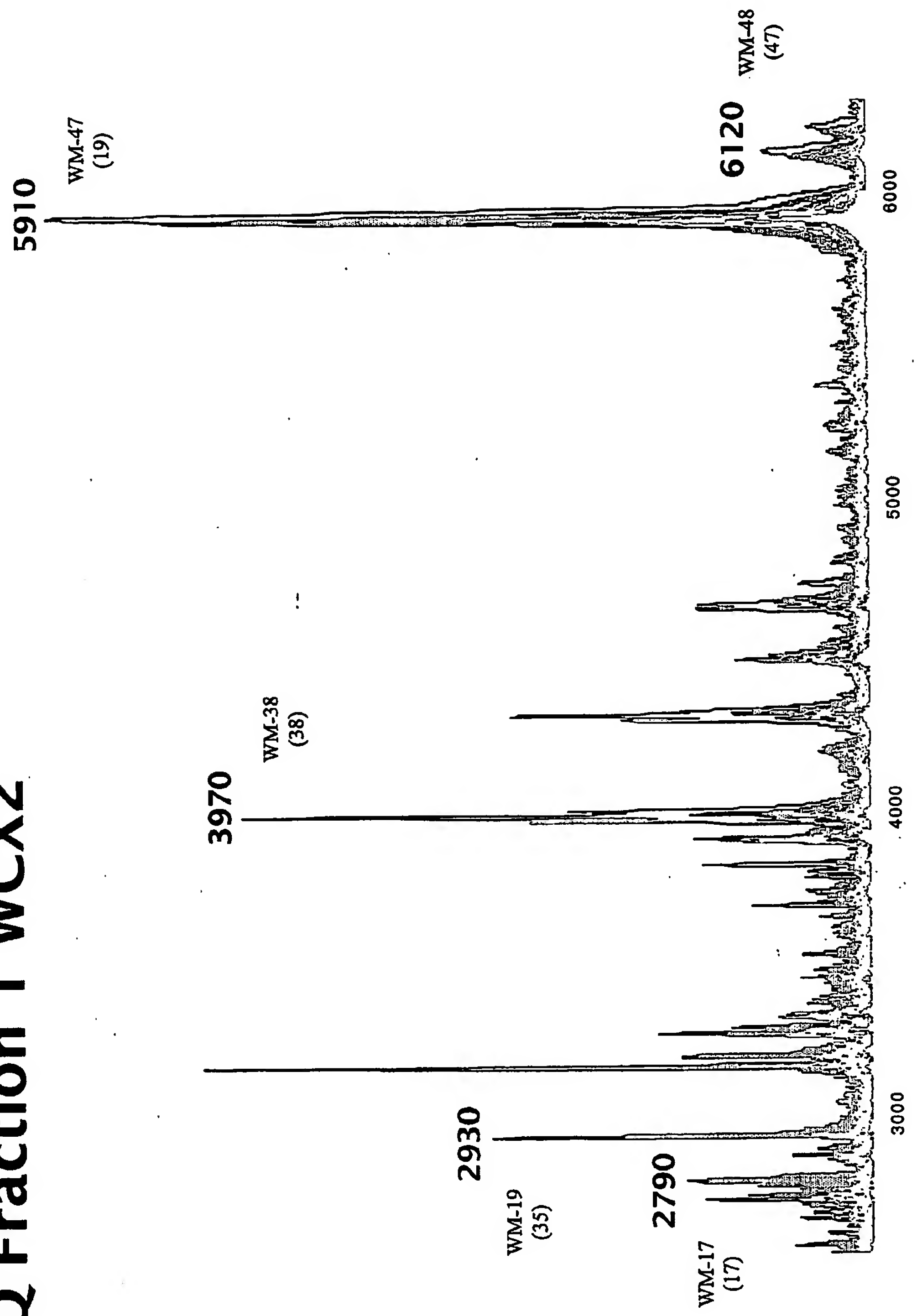


Figure 9
Protein Profile of Selected Samples Q Fraction 2 WCX2

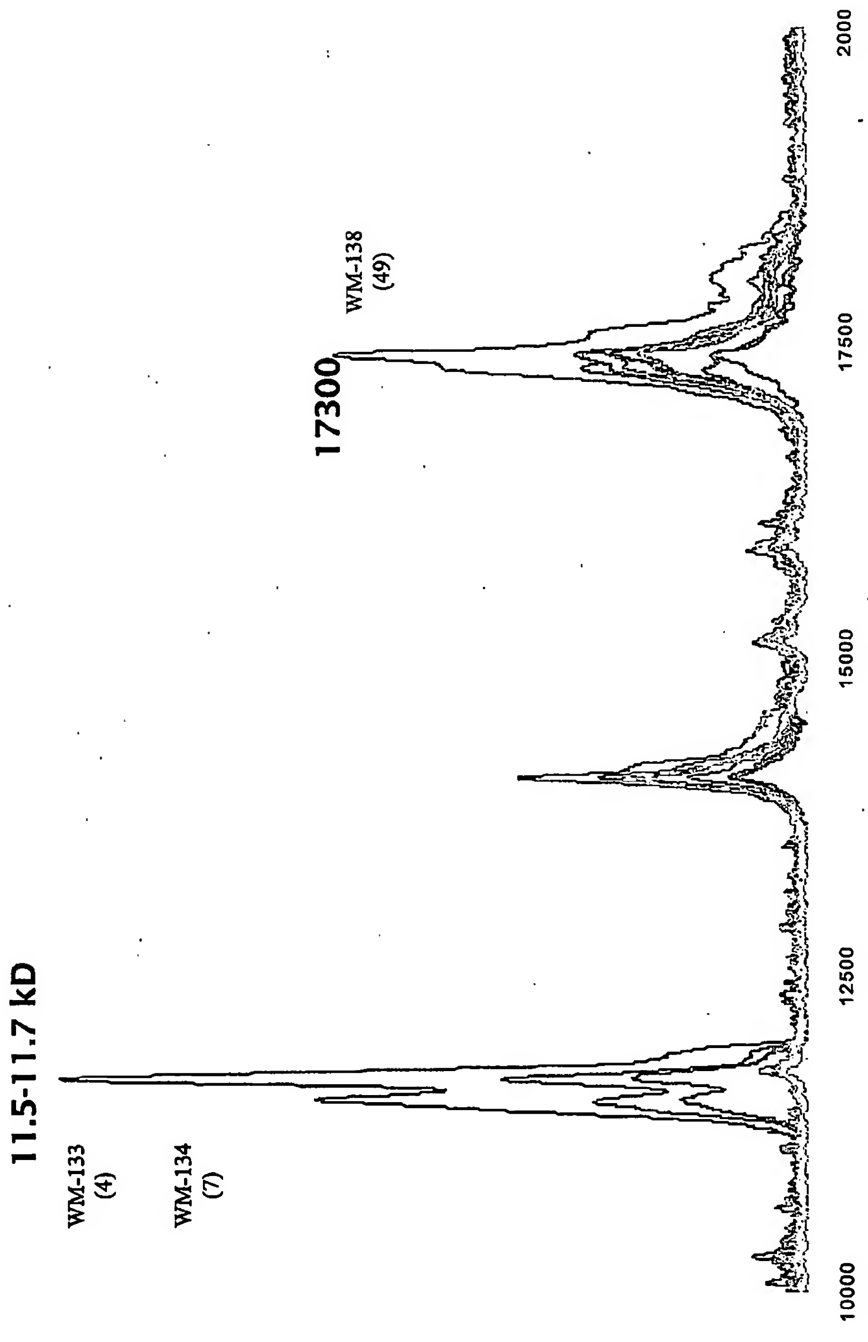


Figure 10
Protein Profile of Selected Samples Q Fraction 2 WCX2

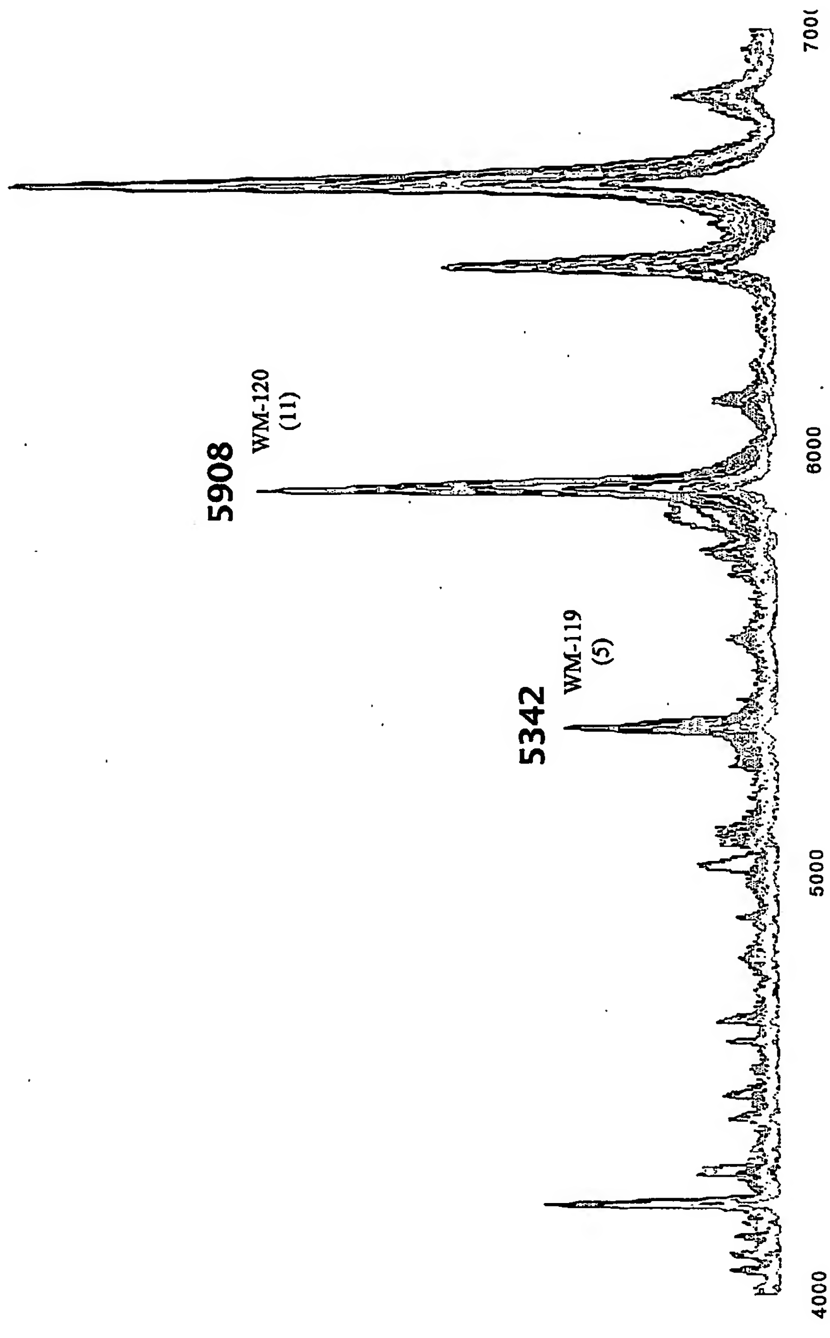


Figure 11
Protein Profile of Selected Samples
Q Fraction 4 WCX2

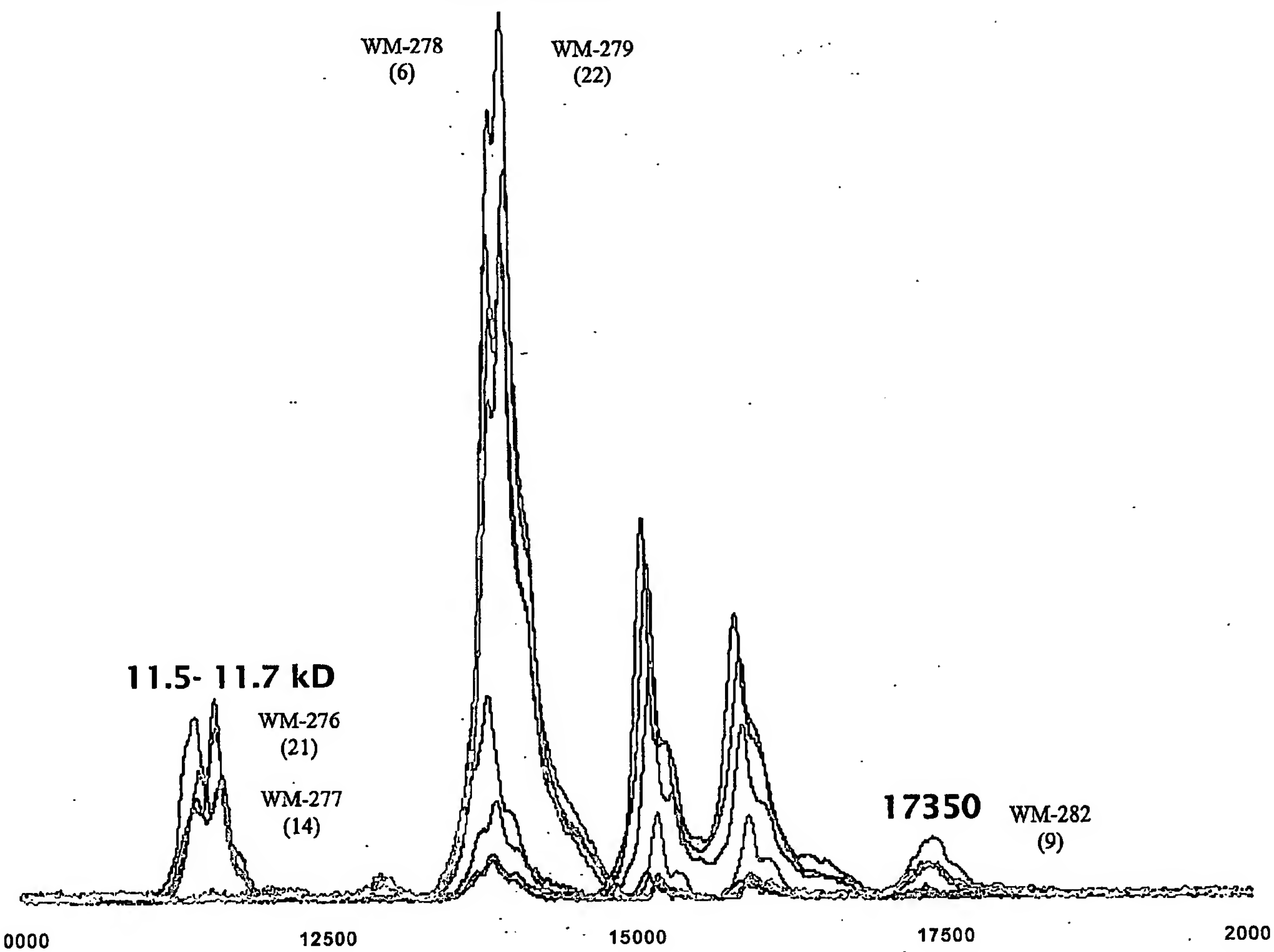


Figure 12
Protein Profile of Selected Samples
Q Fraction 4 WCX2

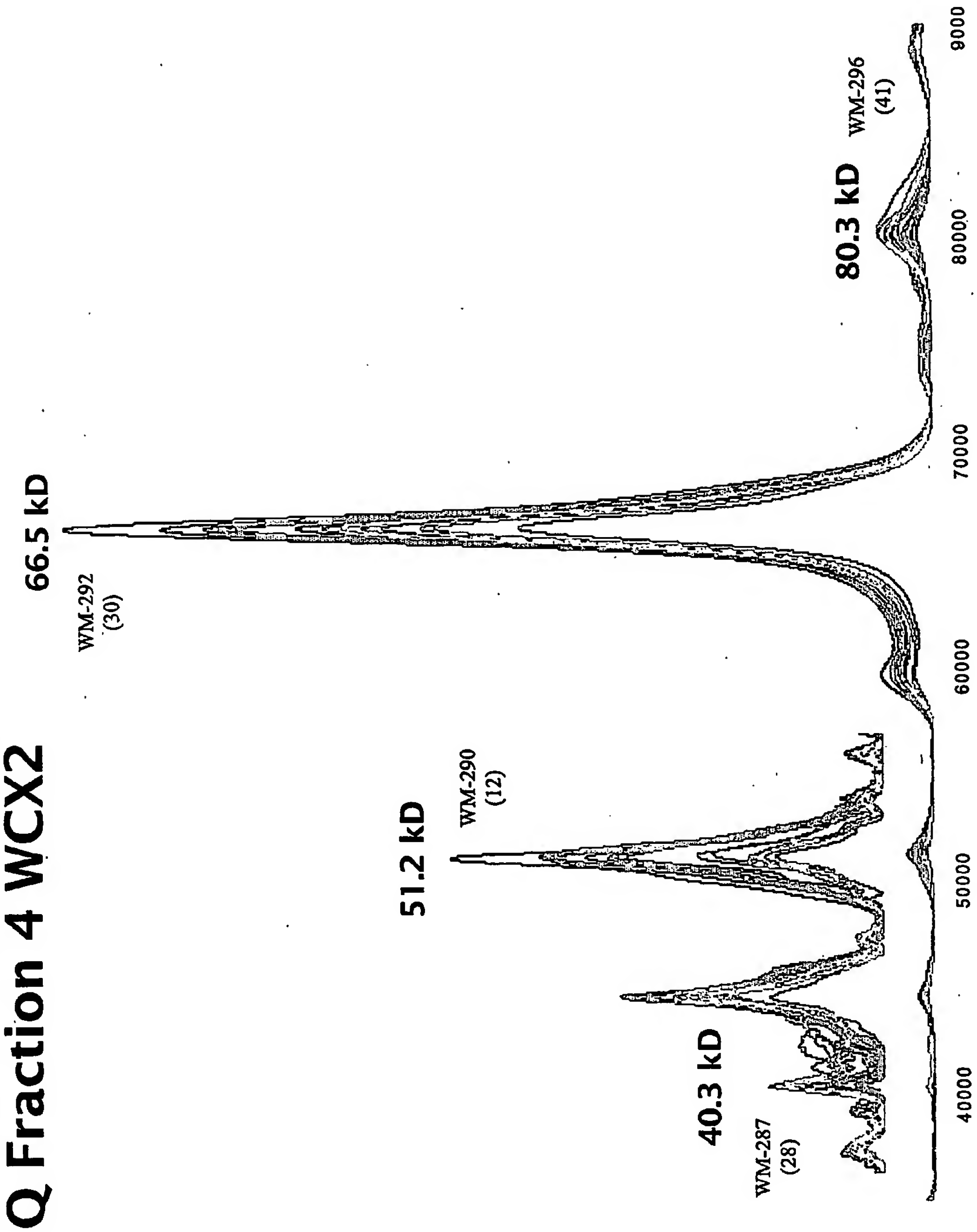


Figure 13
Protein Profile of Selected Samples
Q Fraction 5 WCX2

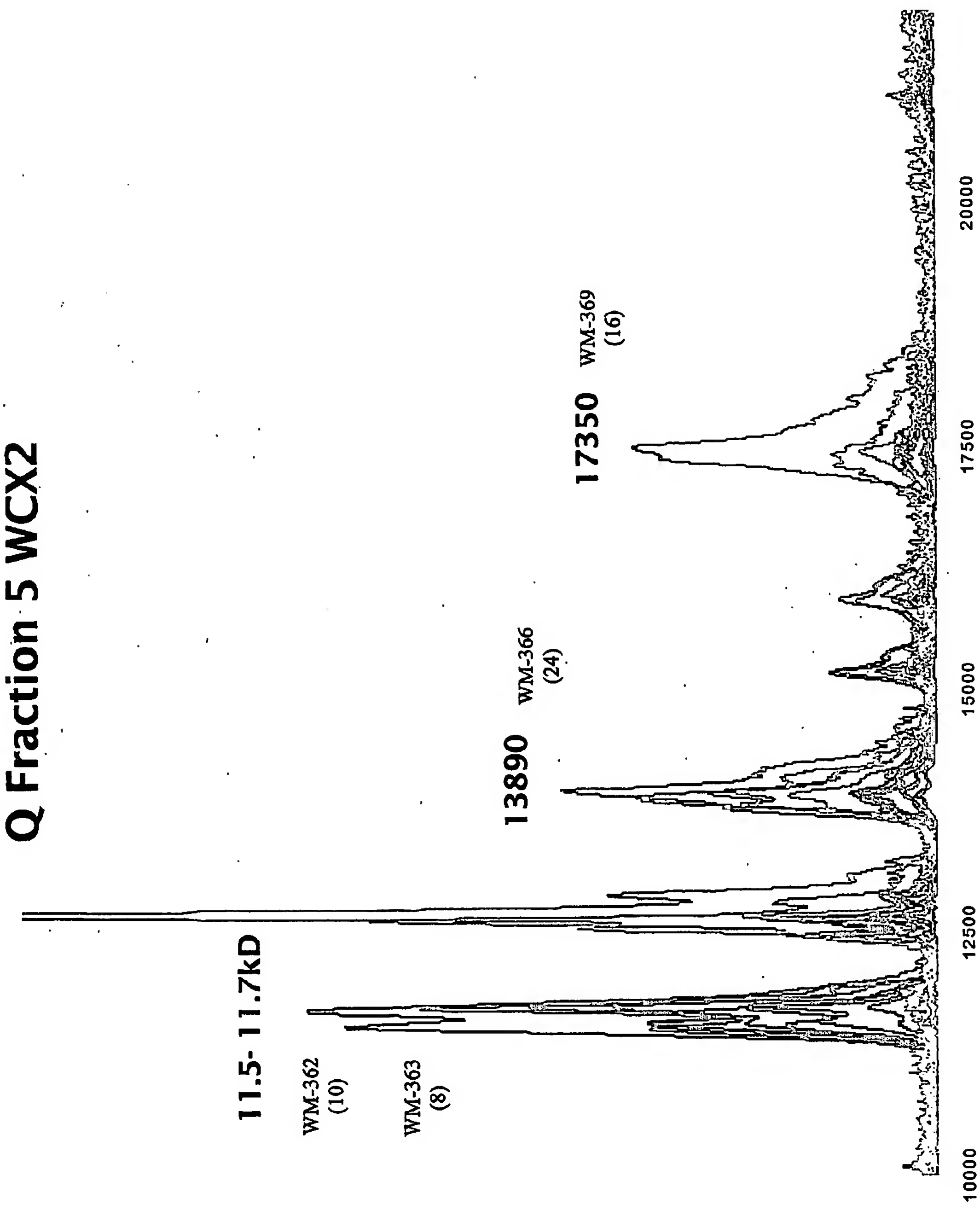


Figure 14
Protein Profile of Selected Samples
Q Fraction 5 WCX2

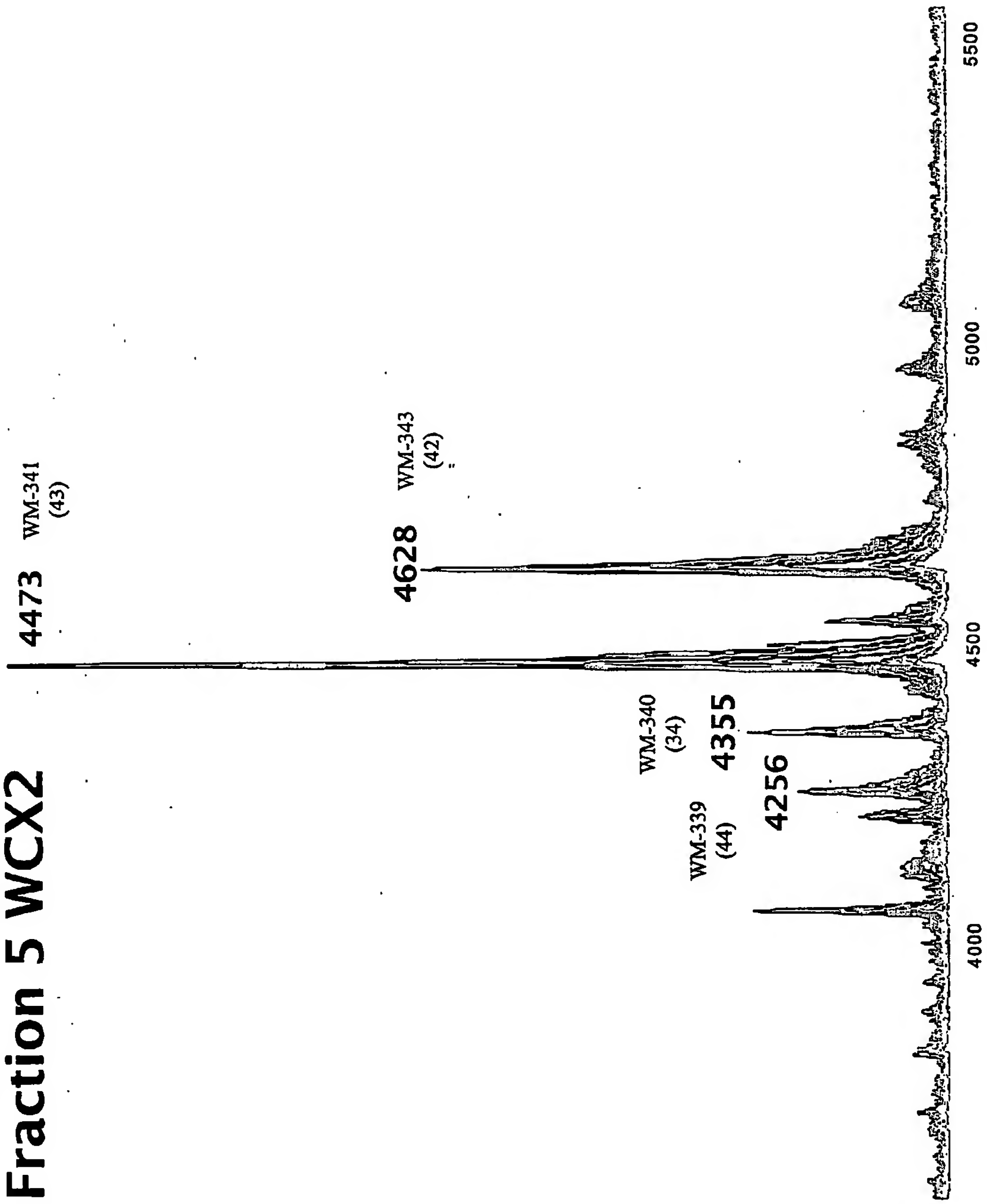


Figure 15

Protein Profile of Selected Samples

Q Fraction 6 WCX2

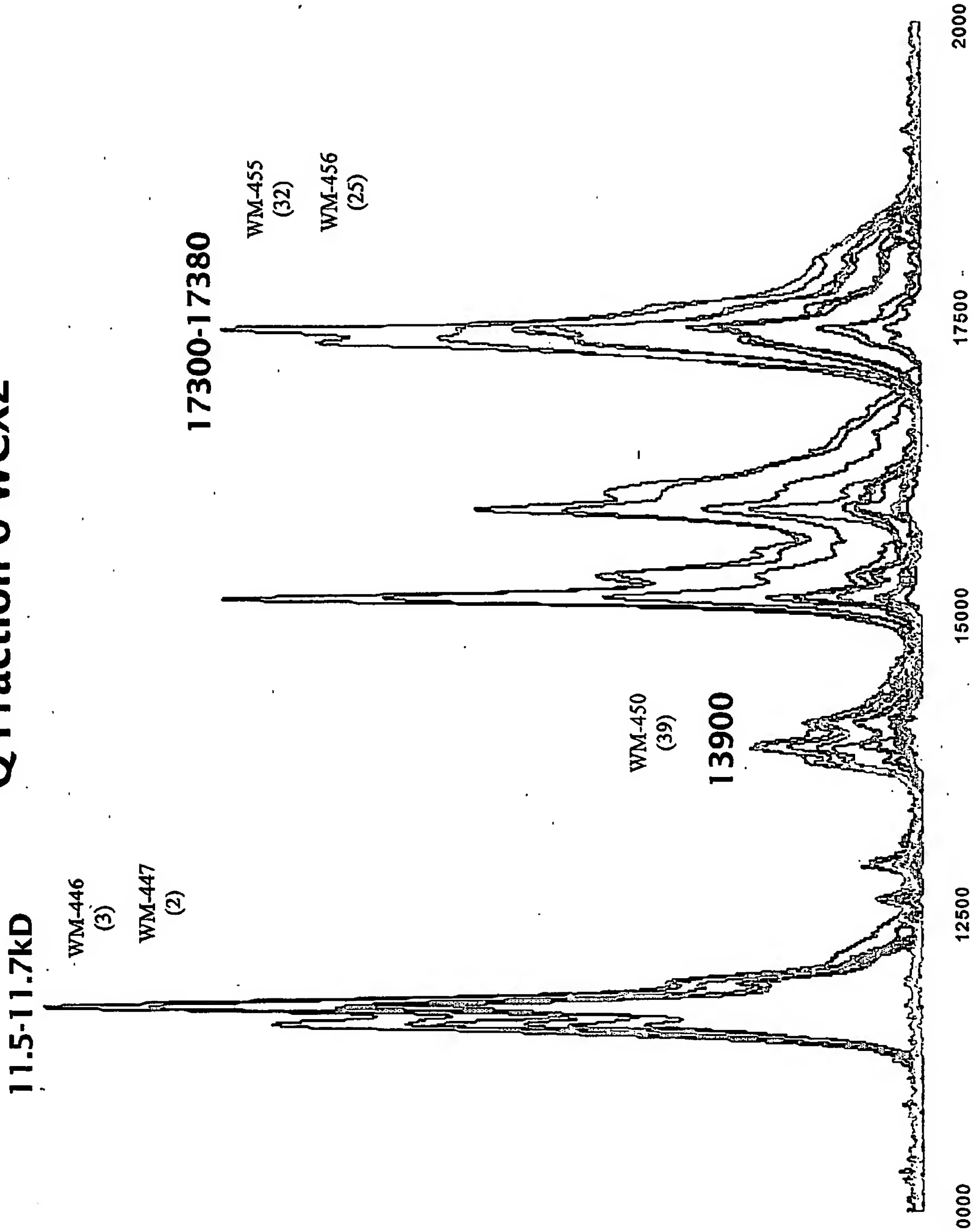


Figure 16

Protein Profile of Selected Samples Q Fraction 6 WCX2

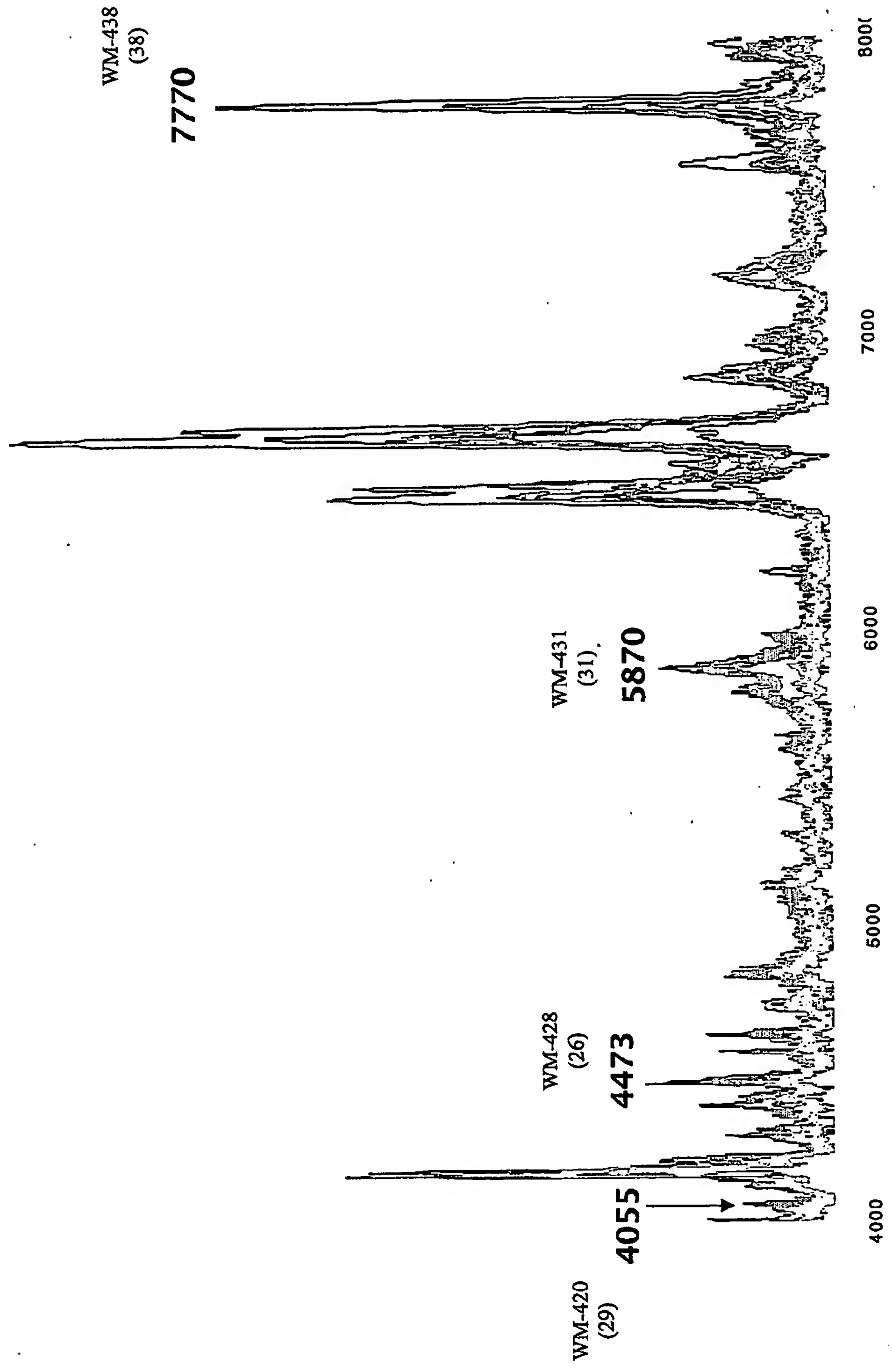


Figure 17
Protein Profile of Selected Samples Q Fraction 2 IMAC-Cu(II)

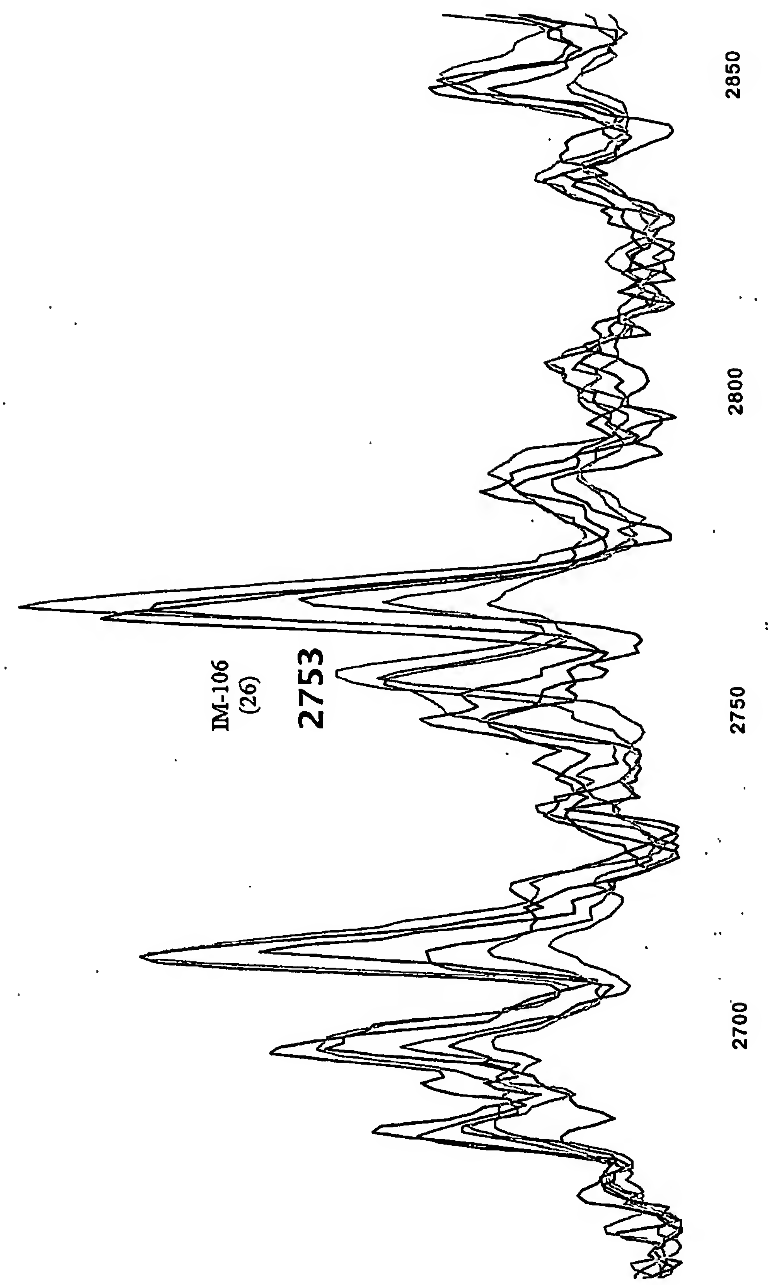


Figure 18
Protein Profile of Selected Samples Q Fraction 2 IMAC-Cu(II)

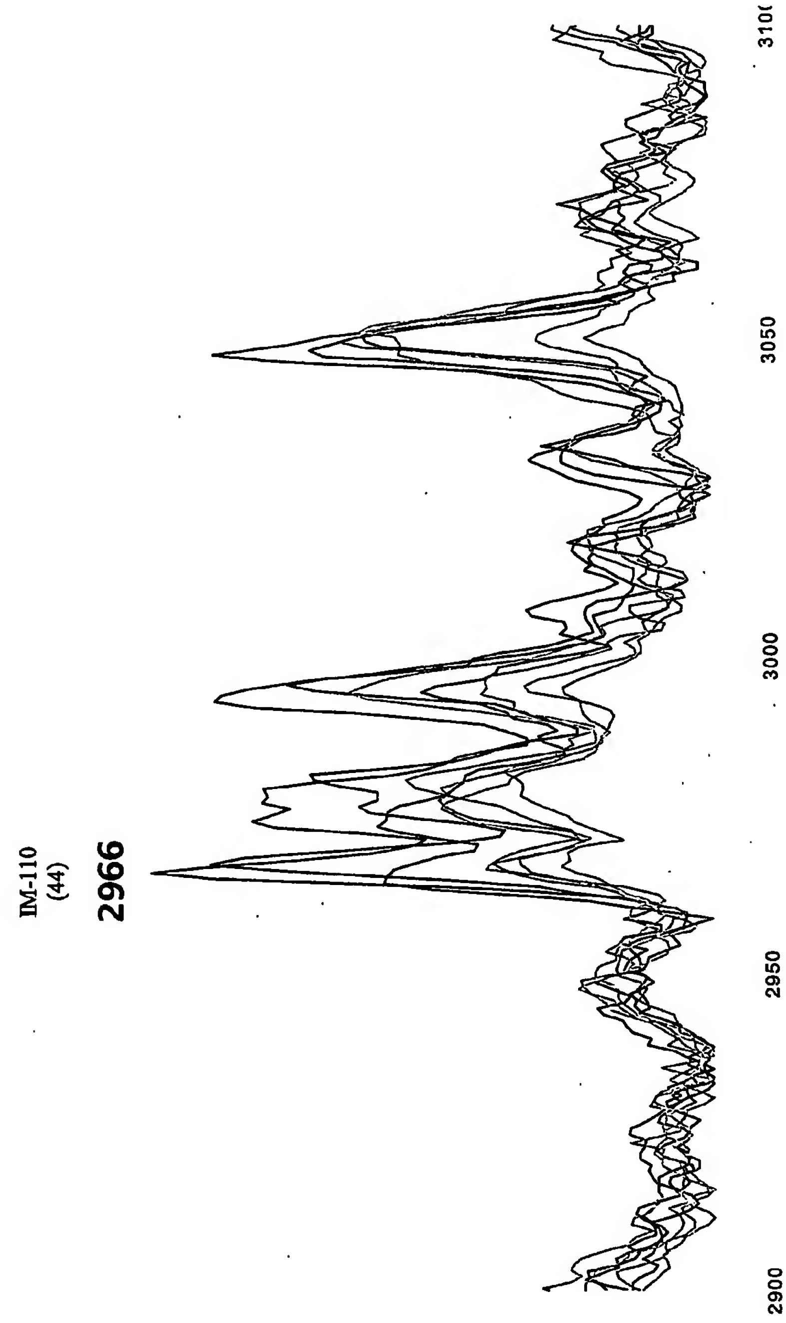


Figure 19
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

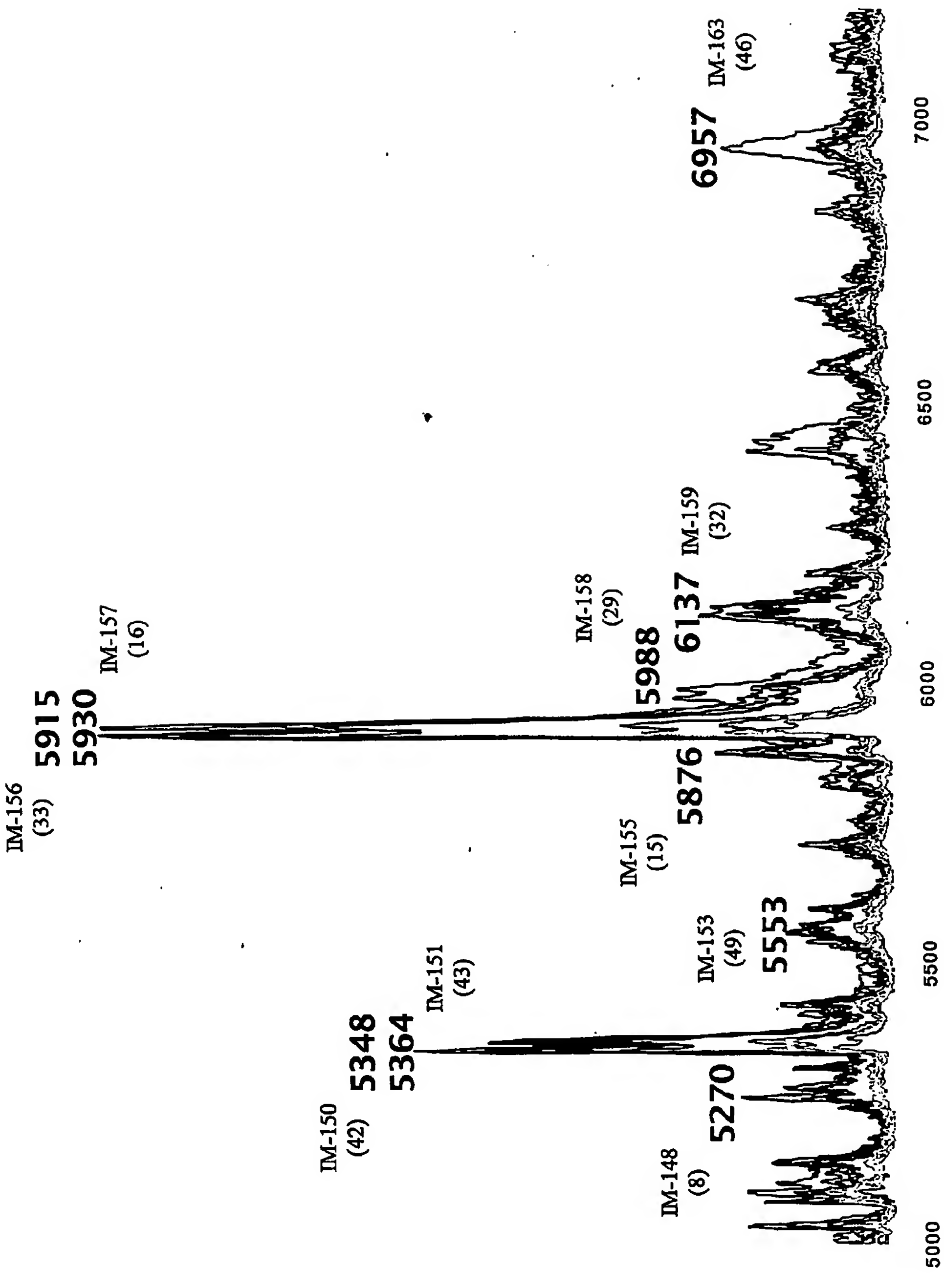


Figure 20
Protein Profile of Selected Samples
Q Fraction 2 IMAC-Cu(II)

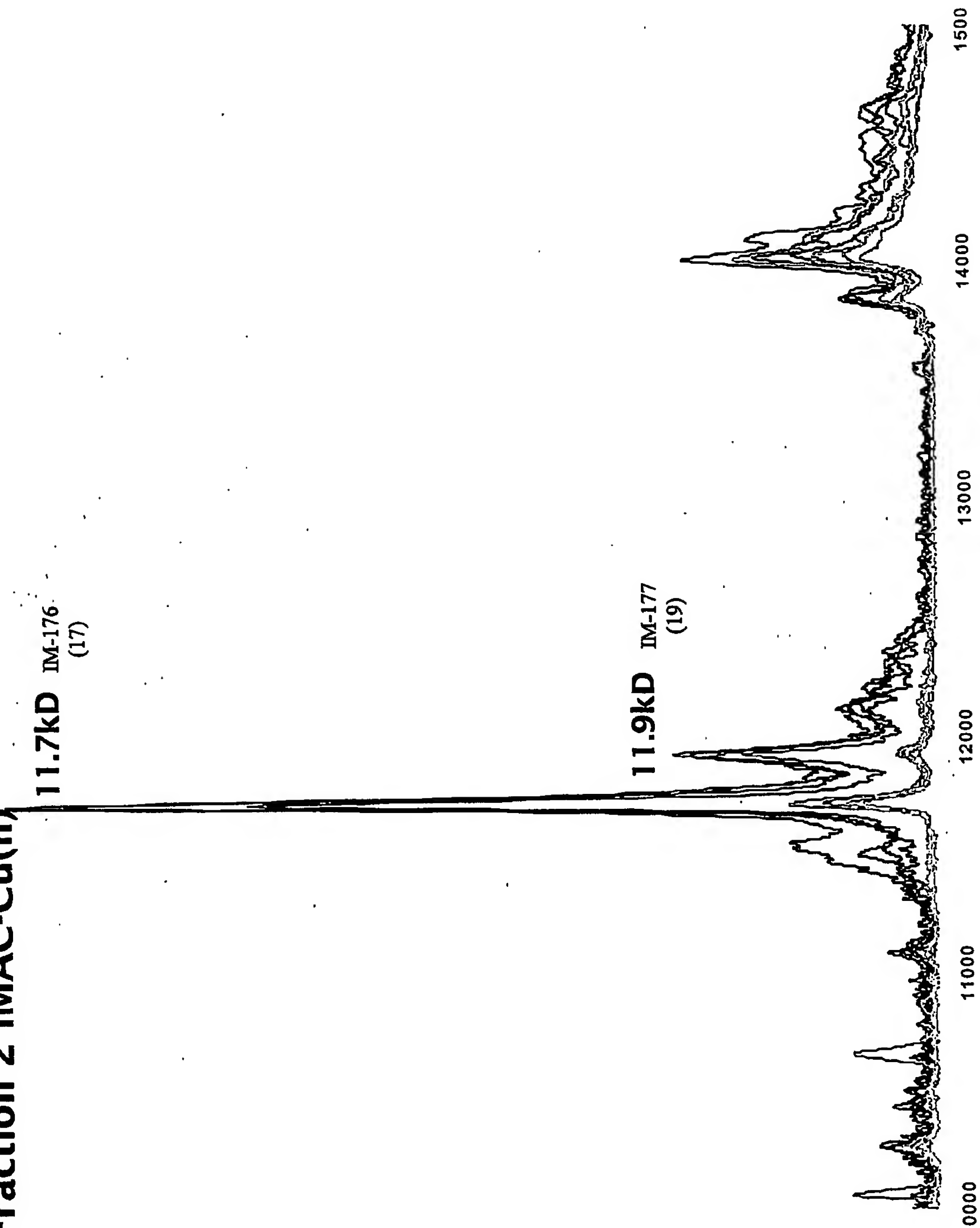


Figure 21

Protein Profile of Selected Samples Q Fraction 3 IMAC-Cu(II)

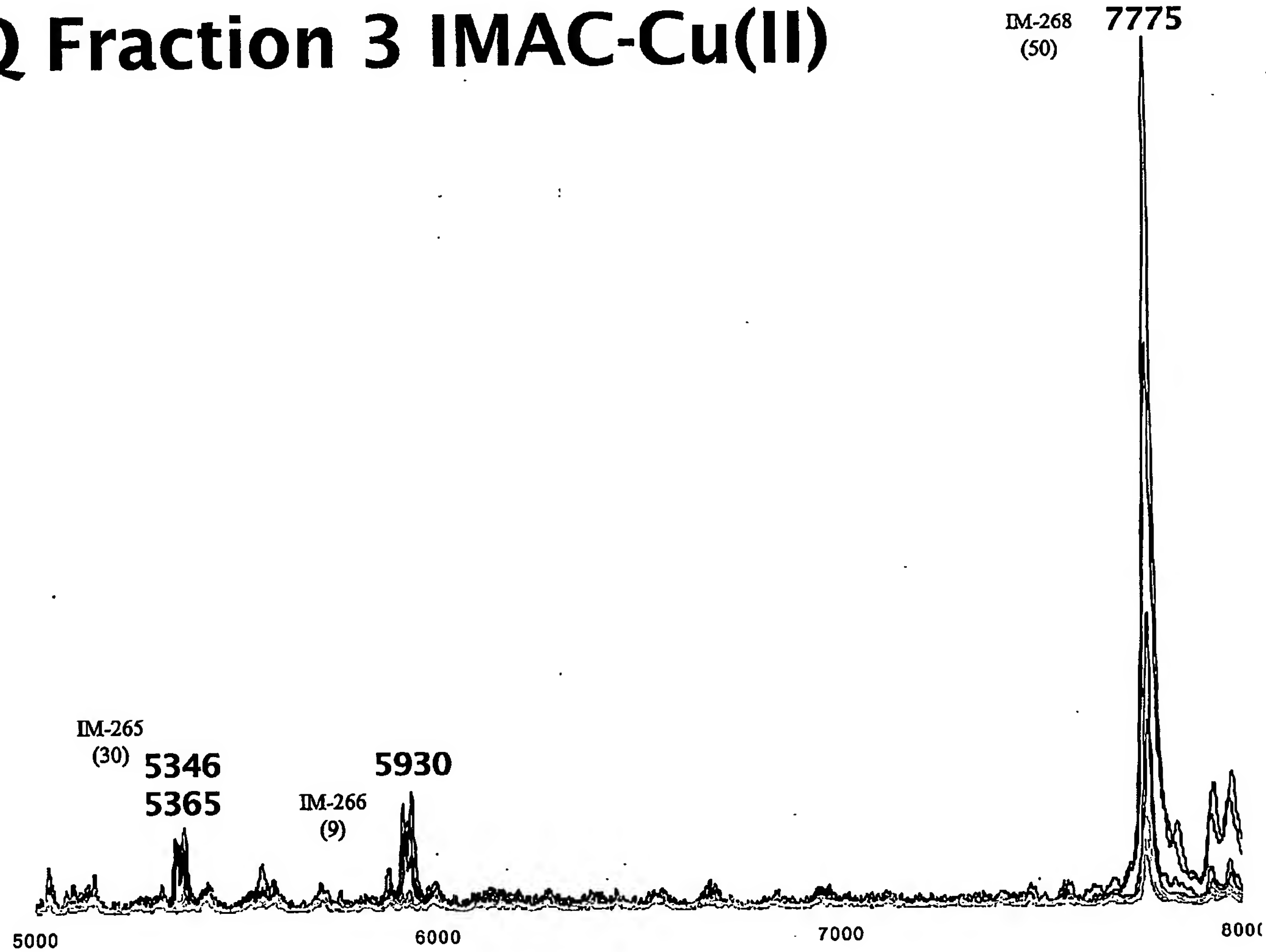


Figure 22

Protein Profile of Selected Samples Q Fraction 3 IMAC-Cu(II)

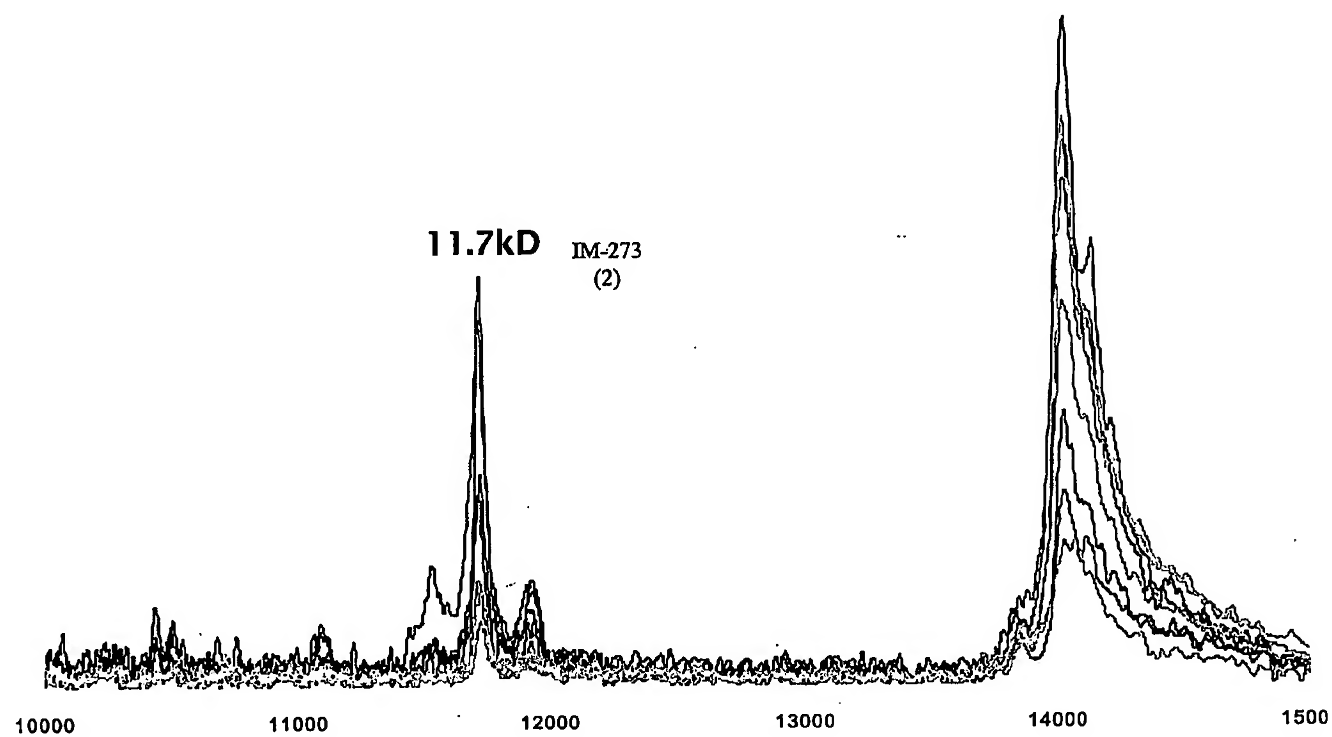


Figure 23
Protein Profile of Selected Samples
Q Fraction 5 IMAC-Cu(II)

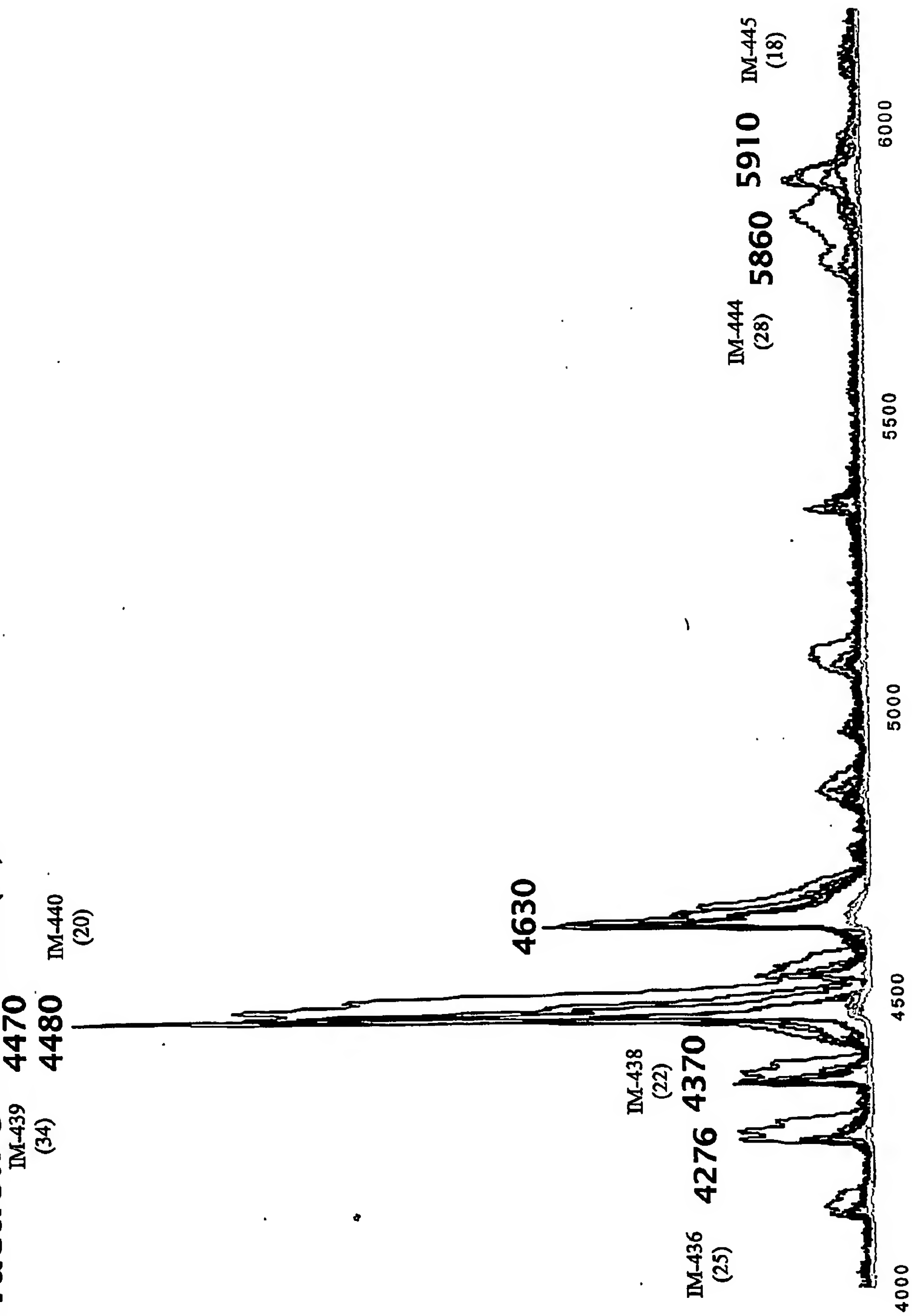


Figure 24
Protein Profile of Selected Samples Q Fraction 5 IMAC-Cu(II)

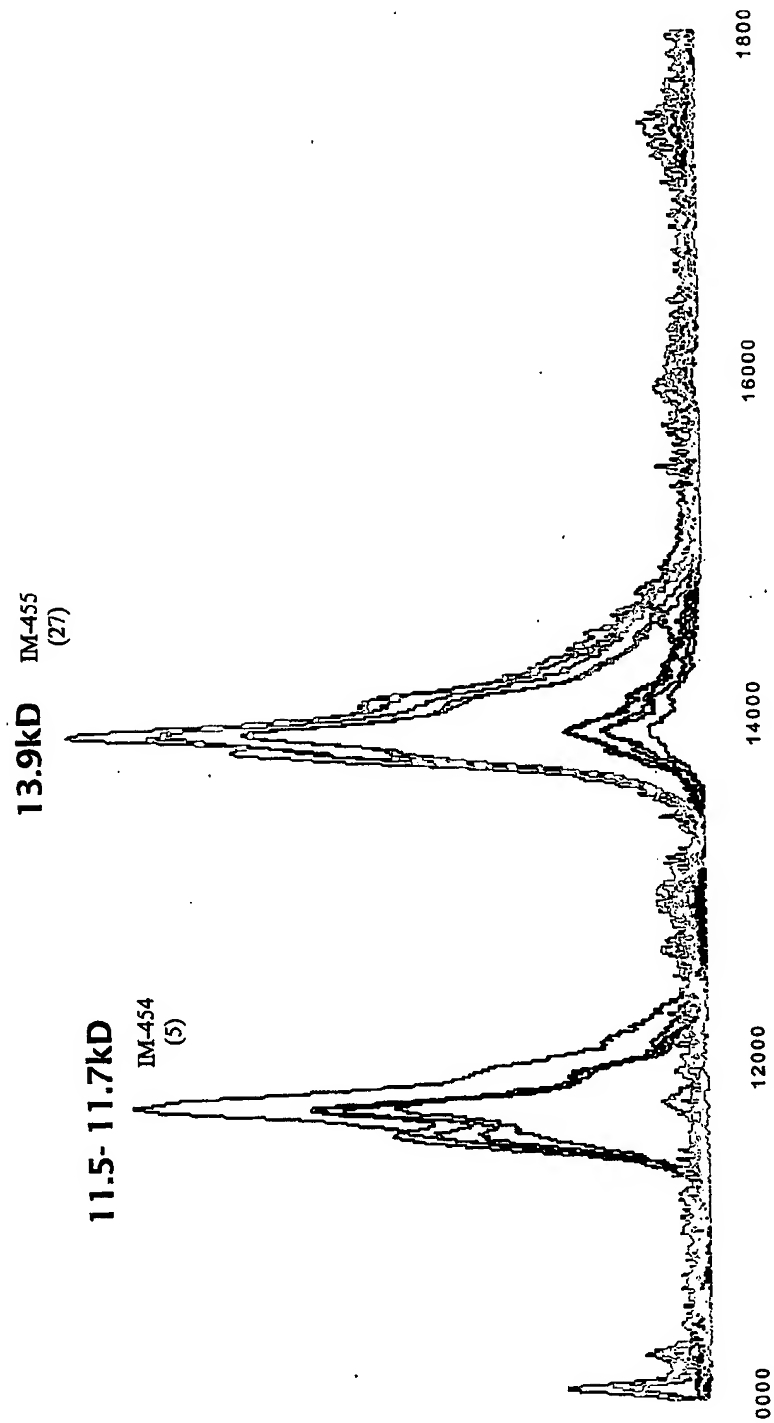


Figure 25
Protein Profile of Selected Samples Q Fraction 5 IMAC-Cu(II)

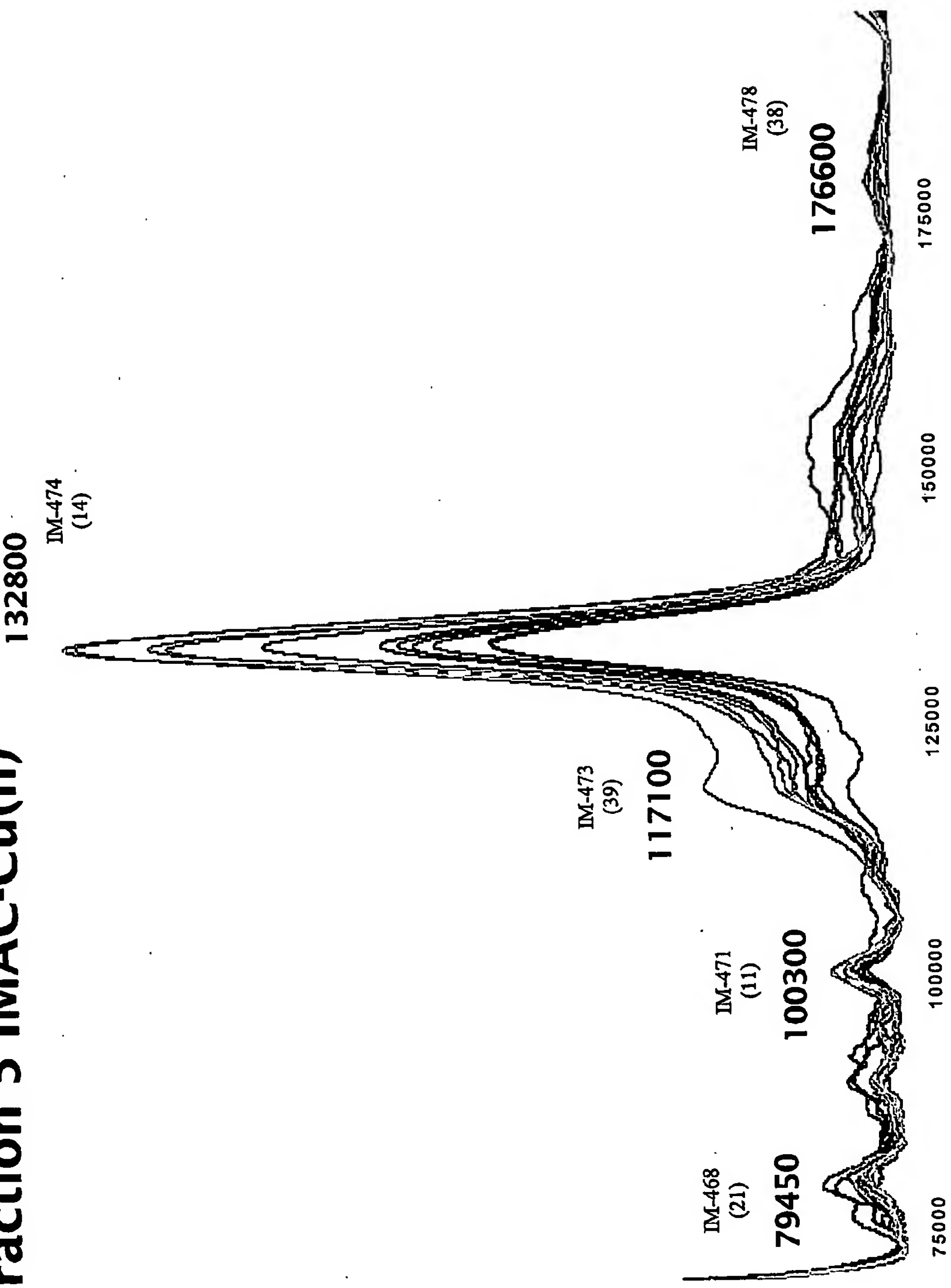
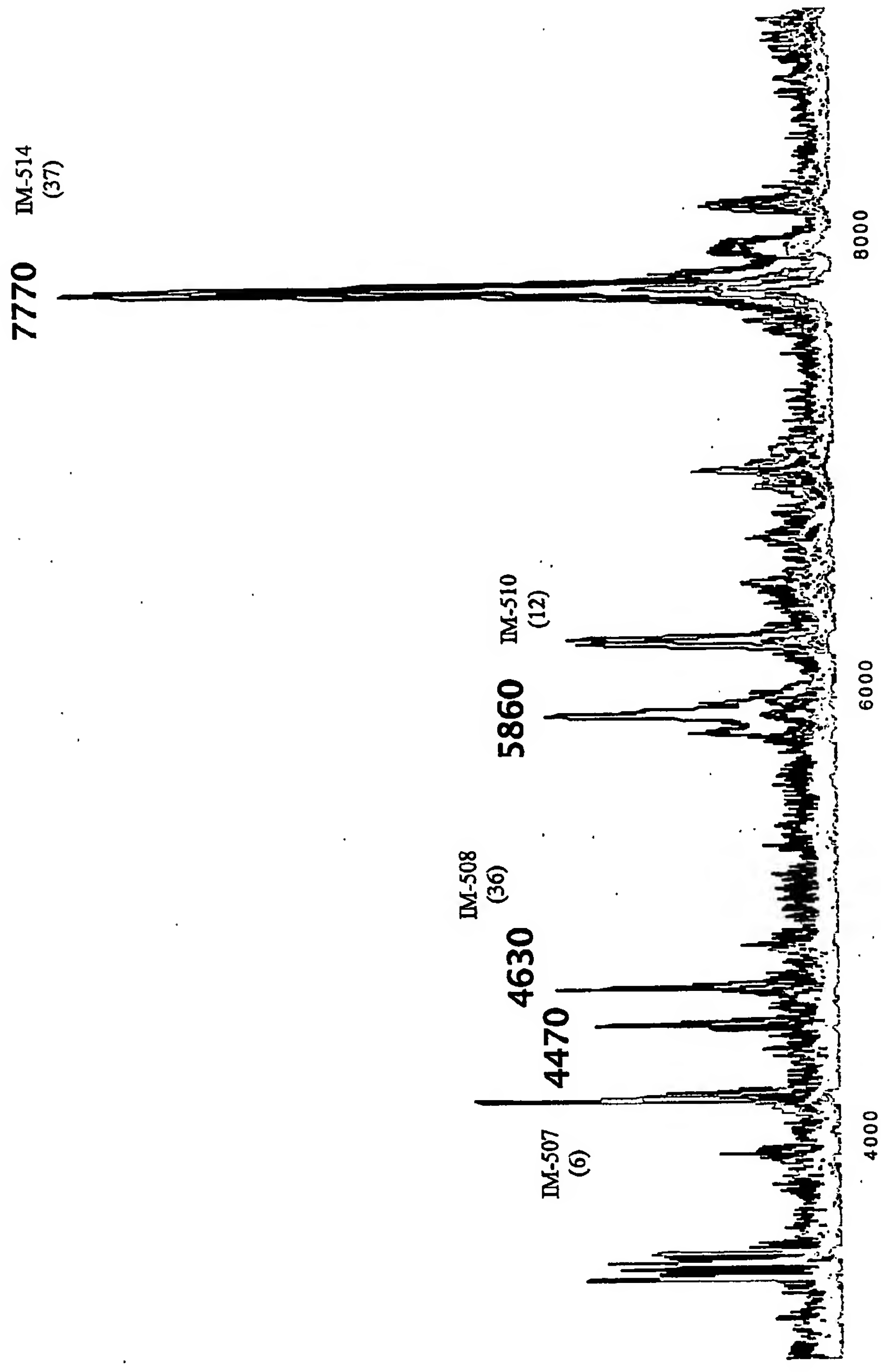


Figure 26
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)



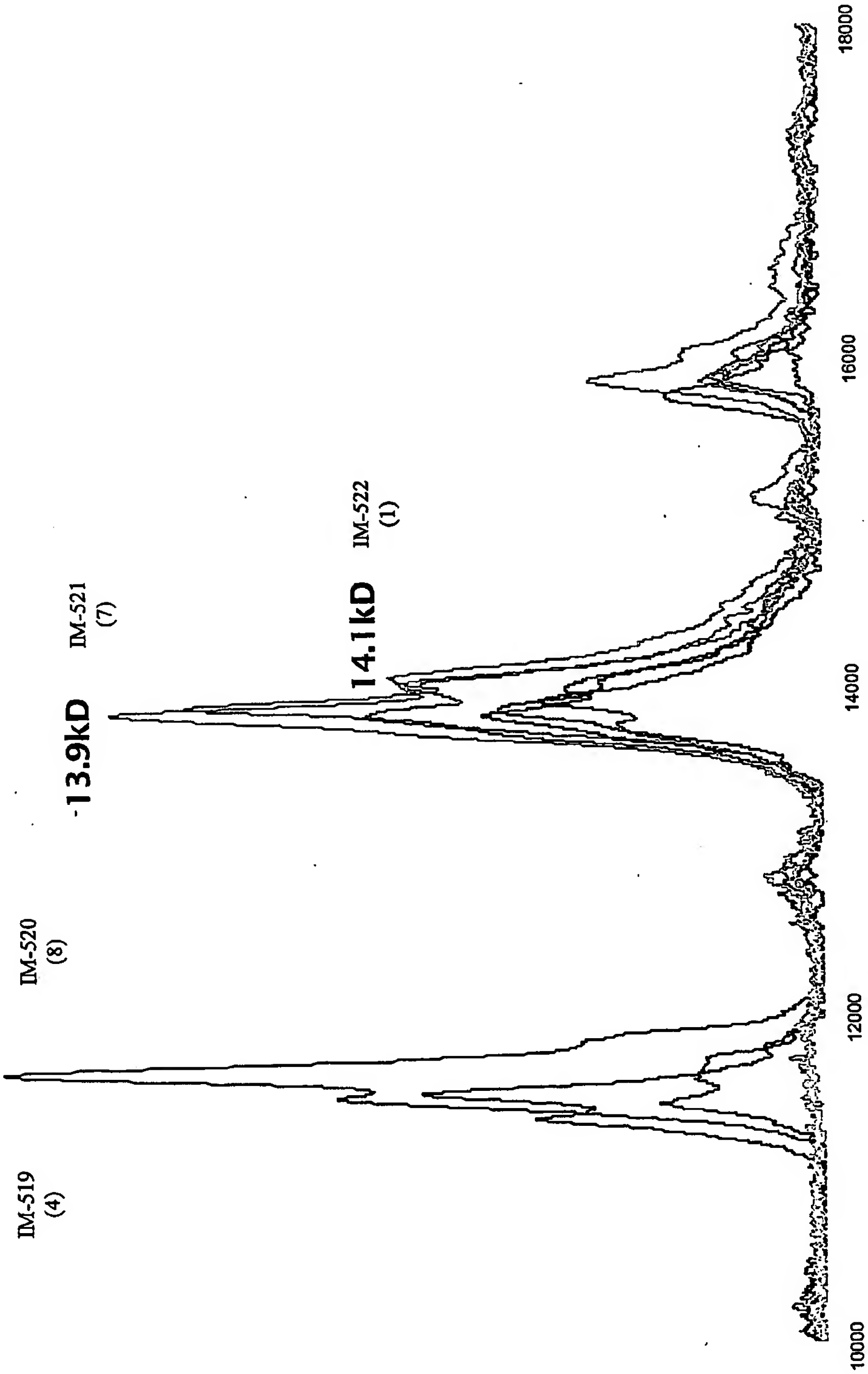
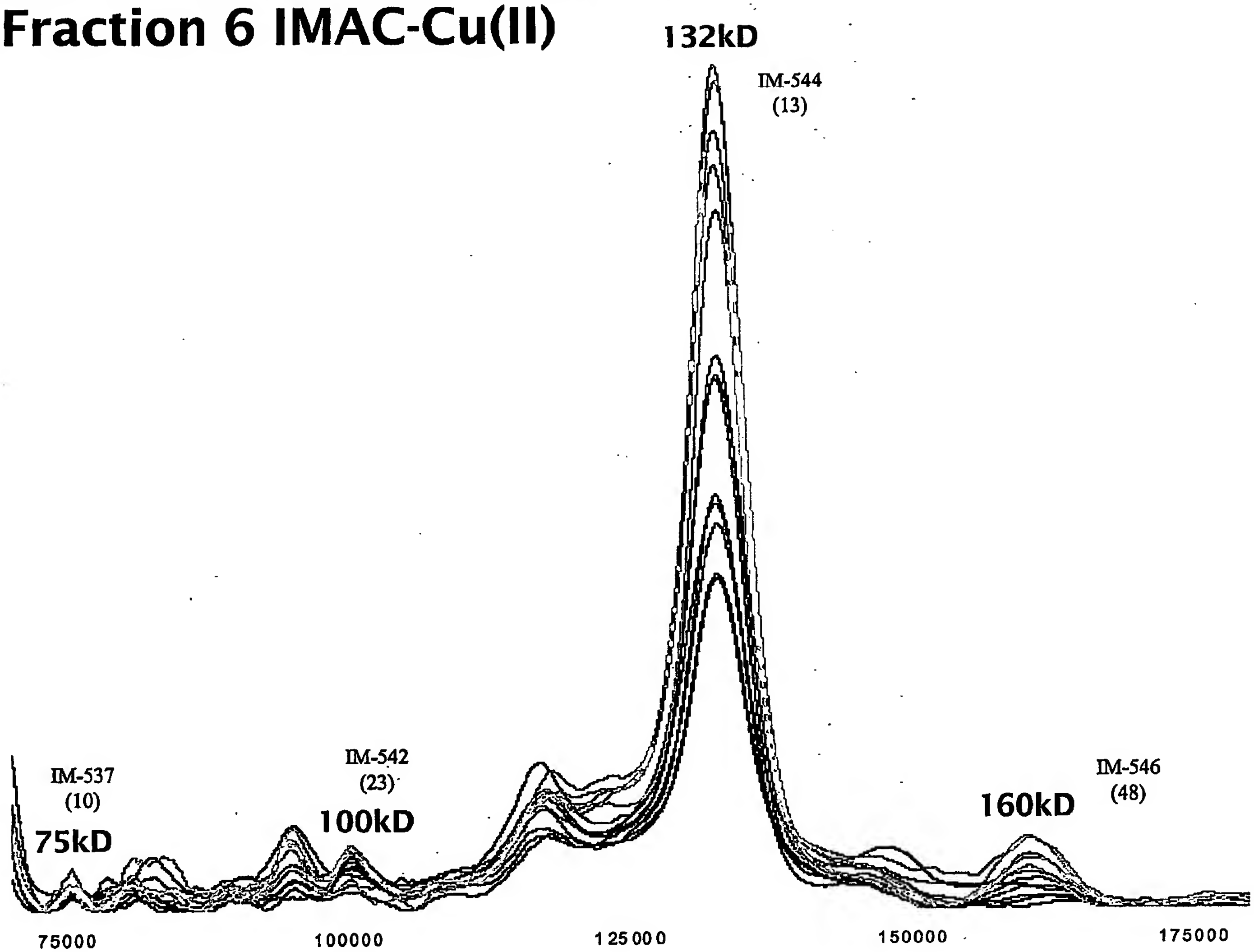


Figure 27
Protein Profile of Selected Samples
Q Fraction 6 IMAC-Cu(II)

Figure 28
Protein Profile of Selected Samples Q Fraction 6 IMAC-Cu(II)



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(57) Abstract: Certain biomarkers and biomarker combinations are useful in a qualifying lung cancer status in a subject. A diagnostic methodology employing these biomarkers and combinations can detect whether a subject has lung cancer.

INTERNATIONAL SEARCH REPORT

International application No.
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IPC(7) : C12Q 1/00; G01N 33/53; A61K 49/00
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According to International Patent Classification (IPC) or to both national classification and IPC

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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